

=> fil reg; e cleavase bn/cn 5
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-key terms

E1	1	CLEAVAMINE-18-CARBOXYLIC ACID, METHYL ESTER, MONOACETA TE/CN
E2	1	CLEAVASE BB/CN
E3	1 -->	CLEAVASE BN/CN
E4	1	CLEAVASE BX/CN
E5	1	CLEAVELANDITE/CN

=> e thermus aquaticus dna polymerase/cn 5

E1	1	THERMPHOS SPR/CN
E2	1	THERMTEX/CN
E3	0 -->	THERMUS AQUATICUS DNA POLYMERASE/CN
E4	1	THERMUS AQUATICUS EXTRACELLULAR SERINE PROTEINASE/CN
E5	1	THERMUS THERMOPHILUS EXTRACELLULAR ENDONUCLEASE/CN

=> e dna polymerase, thermus aquaticus/cn 5

E1	1	DNA POLYMERASE III SUBUNIT .PSI. (ESCHERICHIA COLI GEN E HOLD)/CN
E2	1	DNA POLYMERASE III SUBUNIT .THETA. (ESCHERICHIA COLI G ENE HOLE)/CN
E3	0 -->	DNA POLYMERASE, THERMUS AQUATICUS/CN
E4	1	DNA POLYMERASE-.ALPHA. CATALYTIC SUBUNIT P180 (MOUSE)/ CN
E5	1	DNA POLYMERASE-.ALPHA.-PRIMASE SUBUNIT P68 (CALF THYMU S N-TERMINAL FRAGMENT)/CN

=> e escherichia coli exo iii/cn 5

E1	1	ESCHERICHIA COLI ENDONUCLEASE II/CN
E2	1	ESCHERICHIA COLI ENDONUCLEASE III/CN
E3	0 -->	ESCHERICHIA COLI EXO III/CN
E4	1	ESCHERICHIA COLI EXO-RNASE II/CN
E5	1	ESCHERICHIA COLI EXONUCLEASE I/CN

=> e escherichia coli exonuclease iii/cn 5

E1	1	ESCHERICHIA COLI EXONUCLEASE I/CN
E2	1	ESCHERICHIA COLI EXONUCLEASE II/CN
E3	1 -->	ESCHERICHIA COLI EXONUCLEASE III/CN
E4	1	ESCHERICHIA COLI EXONUCLEASE IV/CN
E5	1	ESCHERICHIA COLI EXONUCLEASE V/CN

=> e saccharomyces cerevisiae radl/cn 5
 E1 1 SACCHAROMYCES CEREVISIAE METALLOPROTEINASE/CN
 E2 1 SACCHAROMYCES CEREVISIAE PROTEINASE A/CN
 E3 0 --> SACCHAROMYCES CEREVISIAE RAD1/CN
 E4 1 SACCHAROMYCES CEREVISIAE SITE-SPECIFIC ENDODEOXYRIBONU
 E5 1 CLAVASE SCE I/CN
 1 CLAVASE BN/CN
 1 ESCHERICHIA COLI EXONUCLEASE III/CN
 2 (CLAVASE BN OR ESCHERICHIA COLI EXONUCLEASE III)/CN
 L1

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=> s cleav? or enzyme# or nuclease# or (aquaticus or thermophil?) (w) (dna
 or deoxyribonucleic) (lw) polymerase# or coli(w) (exoil or exo? or exo? (w) (3
 or iii) or cerevis?) (w) ((radl or rad 1) (a) (radlo or rad 10))
 UNMATCHED LEFT PARENTHESES 'W) (EXOIII'
 The number of right parentheses in a query must be equal to the
 number of left parentheses.

=> s cleav? or enzyme# or nuclease# or (aquaticus or thermophil?) (w) (dna
 or deoxyribonucleic) (lw) polymerase# or coli(w) (exoil or exo? or exo? (w) (3
 or iii) or cerevis?) (w) ((radl or rad 1) (a) (radlo or rad 10))
 L2 655957 FILE CA
 L3 659967 FILE CAPUS

TOTAL FOR ALL FILES
 L4 1315924 CLAV? OR ENZYME# OR NUCLEASE# OR (AQUATICUS OR THERMOPHIL
 ?) (W) (DNA OR DEOXYRIBONUCLEIC) (1W) POLYMERASE# OR COLI(W) (EXOI
 EXOIII OR EXO3 OR EXO2(W) (3 OR III)) OR CEREVIS? (W) ((RAD1
 OR RAD 1) (A) (RAD10 OR RAD 10))

=> fil reg
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=> fil reg; d que l1; fil ca,caplu
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TSCA INFORMATION NOW CURRENT THROUGH DECEMBER 1995

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conducting SmartSELECT searches.

L1 2 SEA FILE=REGISTRY (CLEAVASE BN OR ESCHERICHIA COLI EXONUC
LEASE III)/CN

FILE 'CA' ENTERED AT 16:30:09 ON 08 MAY 96
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=> s l1 or cleavase(1w)bn or (aquaticus or thermophil?)(w)(dna or
deoxyribonucleic)(1w)polymerase# or coli(w)(exoiii or exo3 or exo?(w)(3 or
iii)) or cerevis?(w)((rad1 or rad 1)(a)(rad10 or rad 10))
L2 693 FILE CA
L3 696 FILE CAPLUS

TOTAL FOR ALL FILES

L4 1389 L1 OR CLEAVASE(1W) BN OR (AQUATICUS OR THERMOPHIL?)(W)(DNA
OR DEOXYRIBONUCLEIC)(1W) POLYMERASE# OR COLI(W)(EXOIII OR
EXO3 OR EXO?(W)(3 OR III)) OR CEREVIS?(W)((RAD1 OR RAD 1)
(A)(RAD10 OR RAD 10))

=> fil reg; e dutp/cn 5
FILE 'REGISTRY' ENTERED AT 16:31:55 ON 08 MAY 96
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DICTIONARY FILE UPDATES: 7 MAY 96 HIGHEST RN 175889-01-7

TSCA INFORMATION NOW CURRENT THROUGH DECEMBER 1995

Please note that search-term pricing does apply when
conducting SmartSELECT searches.

E1 1 DUTOM/CN

E2	1	DUTOMYCIN/CN
E3	1	--> DUTP/CN
E4	1	DUTP-21-SS-BIOTIN/CN
E5	1	DUTPASE (EQUINE HERPES VIRUS TYPE 4 CLONE PUC4S GENE B 3)/CN

=> s e3

1 DUTP/CN

=> e "7-deaza-datp"/cn 5

E1	1	7-DEAZA-8-AZAGUANOSINE/CN
E2	1	7-DEAZA-AMP/CN
E3	0	--> 7-DEAZA-DATP/CN
E4	1	7-DEAZADENINE/CN
E5	1	7-DEAZAADENOSINE/CN

=> e "7-deaza-deoxyadenosine triphosphate"/cn 5

E1	1	7-DEAZA-8-AZAGUANOSINE/CN
E2	1	7-DEAZA-AMP/CN
E3	0	--> 7-DEAZA-DEOXYADENOSINE TRIPHOSPHATE/CN
E4	1	7-DEAZADENINE/CN
E5	1	7-DEAZAADENOSINE/CN

=> e "7-deazadeoxyadenosine triphosphate"/cn 5

E1	1	7-DEAZADENOSINE-7-CARBOXAMIDE/CN
E2	1	7-DEAZADEOXYADENOSINE/CN
E3	0	--> 7-DEAZADEOXYADENOSINE TRIPHOSPHATE/CN
E4	1	7-DEAZAGUANINE/CN
E5	1	7-DEAZAGUANOSINE/CN

=> e "7-deazadeoxyguanosine triphosphate"/cn 5

E1	1	7-DEAZADENOSINE-7-CARBOXAMIDE/CN
E2	1	7-DEAZADEOXYADENOSINE/CN
E3	0	--> 7-DEAZADEOXYADENOSINE TRIPHOSPHATE/CN
E4	1	7-DEAZAGUANINE/CN
E5	1	7-DEAZAGUANOSINE/CN

=> e "7-deaza-dgtp"/cn 5

E1	1	7-DEAZA-8-AZAGUANOSINE/CN
E2	1	7-DEAZA-AMP/CN
E3	0	--> 7-DEAZA-DGTP/CN
E4	1	7-DEAZADENINE/CN
E5	1	7-DEAZAADENOSINE/CN

=> fil ca,caplus

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=> s 14 and (15 or 7(w)deaza? or dntp or d utp or deoxyuridinetriphosphate or deoxyuridine? or deoxy uridine?)

L6 15 FILE CA
L7 15 FILE CAPLUS

TOTAL FOR ALL FILES

L8 30 L4 AND (L5 OR 7(W) DEAZA? OR DUTP OR D UTP OR DEOXYURIDINE
TRIPHOSPHATE OR DEOXYURIDINE? OR DEOXY URIDINE?)

=> dup rem l8; d 1-15 .bevstr; fil
biosi,medl,embas,lifesci,biotechds,wpids,confsci,dissabs,scisearch
PROCESSING COMPLETED FOR L8

L9 15 DUP REM L8 (15 DUPLICATES REMOVED)

L9 ANSWER 1 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 1
AN 124:195956 CA
TI PCR with fluorescently labeled nucleotide triphosphates for DNA
sequence determination
IN Ikeda, Katsunori; Inoe, Hiroaki; Oka, Masanori; Kawamura, Yoshihisa
PA Toyo Boseki, Japan
SO Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF
PI JP 07313198 A2 951205 Heisei
AI JP 94-108504 940523
DT Patent
LA Japanese
OS MARPAT 124:195956
AB Disclosed is a method using unlabeled nucleotide triphosphates, DNA
synthetase, and biotin-, digoxigenin-, enzyme- or fluorescent
dye-labeled nucleotide triphosphates for DNA sequence detn. The
nucleotide triphosphate deriv. is e.g. dideoxy-7-
deaza nucleotide-5'-triphosphate, and the labeled nucleotide
triphosphate is e.g. 7-[N-biotinyl-(3-amino-1-propyl)]-2',3'-dideoxy-
7-deazaguanosine-5'-triphosphate,
7-[N-biotinyl-(3-amino-1-propyl)]-2',3'-dideoxy-7-
deazaadenosine-5'-triphosphate, 5-[N-biotinyl-(3-amino-1-
propyl)]-2',3'-dideoxyuridine-5'-triphosphate, and
5-[N-biotinyl-(3-amino-1-propyl)]-2',3'-dideoxycytidine-5'-
triphosphate. Alk. phosphatase, avidin, 1,2-dihydroxycetane,
5-bromo-4-chloro-3-indolyl phosphate, or NitroBlue tetrazolium is
used for signal generation. 5'.fwdarw.3' Exonuclease, 3'.fwdarw.5'
exonuclease, Thermus thermophilus-derived Tth DNA polymerase,
Thermus aquaticus-derived Taq DNA polymerase, and Pyrococcus
furiosus-derived DNA polymerase are usable for the disclosed method.
IT 79393-91-2, 3'.fwdarw.5' Exonuclease
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(PCR with unlabeled nucleotide triphosphates, DNA synthetase, and
biotin-, digoxigenin-, enzyme- or fluorescent dye-labeled
nucleotide triphosphates for DNA sequence detn)

L9 ANSWER 2 OF 15 CA COPYRIGHT 1996 ACS

DUPLICATE 2

AN 122:257225 CA
 TI In situ transcription with Tth DNA polymerase and fluorescent nucleotides
 AU Chang, Henry
 CS Naval Medical Research Institute, Code 63, Bethesda, MD, 20889-5607,
 SO USA
 J. Immunol. Methods (1994), 176(2), 235-43
 CODEN: JIMMBG; ISSN: 0022-1759
 DT Journal
 LA English
 AB We and others have described methods to label specific nucleic acid sequences in fixed cells by reverse in situ transcription (IST). These methods are simple alternatives to the tedious steps of in situ hybridization with labeled probes. We have favored use of thermostable DNA polymerases after heat denaturation of the template secondary structure, accompanied by synthesis of cDNA from an annealed primer, but the approach has been limited by the low reverse transcriptase (RT) activity of Tth polymerase and by delayed detection methods. We have improved the technique by the use of recombinant Thermus thermophilus (Tth) DNA polymerase and fluorescein-12-dUTP (FIST). Jurkat T lymphocytes were stimulated with ionomycin and phorbol myristate acetate to produce interleukin-2 (IL-2) mRNA in vitro overnight. They were cytospun onto slides and fixed in 70% ethanol + 30% DEPC-treated water, acetone, and were air-dried. The slides were placed on a temp.-controlled heating block, and the cell spot was covered with a plastic coverslip. The temp. was raised to 95.degree.C, and 5-10 .mu.l of modified Perkin-Elmer/Cetus Tth RT reaction mix was injected under the edge of the coverslip. Each 10 .mu.l of mix in DEPC-water contained 10 mM Tris-HCl, pH 8.3, 90 mM KCl, 1 mM MnCl2, 1 mM dithiothreitol, 10 U placental RNase inhibitor, 0.125 mM dA,C,GTPs, 0.1 mM fluorescein-12-dUTP, 2 U Tth DNA polymerase, and 4 pM 22-mer oligonucleotide primer, which spanned the second intron of IL-2. After 3 min at 95.degree.C, 1 min at 50.degree.C, and 10 min at 72.degree.C, the slides were washed in 0.5.times. phosphate-buffered saline, pH 7.0 at 42.degree.C, in 70% ethanol, 100% ethanol, and were air-dried. The cells were mounted in antifade soln. (2% n-Pr gallate in 70% glycerol), and could be viewed immediately by fluorescence microscopy. Image anal. showed that stimulated Jurkat cells were brighter than uninduced controls or those treated with RNase or those treated without polymerase or primer. FIST appears to be useful for the detection of specific mRNAs in single cells.

ANSWER 3 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 3

TI Detection of 5-bromo-2-deoxyuridine (BrdUrd) incorporation with monoclonal anti-BrdUrd antibody after deoxyribonuclease treatment

AU Takagi, Shuji; McFadden, Marcia L.; Humphreys, Robert E.; Woda, Bruce A.; Saitenji, Takeshi
 CS Med. Sch., Univ. Massachusetts, Worcester, MA, 01655, USA
 SO Cytometry (1993), 14(6), 640-8

CODEN: CYTODQ; ISSN: 0196-4763

DT Journal

LA English

AB The effects of DNases on the detection of 5-bromo-2-

deoxyuridine (BrdUrd) by anti-BrdUrd monoclonal antibodies

(mAbs) were studied. After DNase I treatment, BrdUrd was detected in cells fixed on slides with the anti-BrdUrd mAbs, B44 and BMC9318. The level of detection related to the degree of DNA digestion. DNA digestion of 25-75% resulted in levels of staining comparable to control preps. in which DNA was denatured by heating with formamide. Staining with the mAbs of DNase I-treated cells was abolished with S1 nuclease, a single-stranded DNA-specific nuclease. When exonuclease III was used after DNase I treatment, the staining intensity of cells fixed on slides increased, and BrdUrd could be detected in suspended cells by flow cytometry. Since this enzymic method leading to the detection of BrdUrd does not involve cell loss, or destruction of either cellular morphol. or epitope reactivity, as occurs with traditional DNA denaturation procedures, it is useful for kinetic studies of phenotypically mixed populations. Furthermore, staining with anti-BrdUrd mAb of cells treated with exonuclease III offers a simple approach to quantitation of apoptotic cells, in which an endogenous endonuclease is activated.

IT 9037-44-9, Exonuclease III

RL: ANST (Analytical study)

(bromodeoxyuridine detection with monoclonal antibodies after DNase treatment in relation to)

L9 ANSWER 4 OF 15 CA COPYRIGHT 1996 ACS

DUPLICATE 4

AN 119:132483 CA

TI Selective digestion of mouse chromosomes with restriction endonucleases. Oligonucleotide priming of single-stranded DNA produced with exonuclease III

AU Gosalvez, J.; Lopez-Fernandez, C.; Garcia de la Vega, C.; Mezzanotte, R.; Fernandez, J. L.; Goyanes, V.

CS Fac. Cienc., Univ. Auton. Madrid, Madrid, 28049, Spain

SO Genome (1993), 36(2), 230-4

CODEN: GENOE3; ISSN: 0831-2796

DT Journal

LA English

AB L-929 mouse chromosomes prepd. for electron microscopy have been treated with MspI, EcoRI, and HaeIII restriction endonucleases (REs). RE-induced nicks were amplified with exonuclease III to obtain single-stranded DNA (ssp-DNA) motifs. The ss-DNA produced was enough to permit hybridization of a series of random oligonucleotides. These can be used as primers, which are extended by the Klenow fragment using non-isotopic labeled dUTP. The incorporation of biotinylated dUTP is detected with a gold-tagged streptavidin as the reporter mol. This allows, in mouse chromosomes, the detection of different rates of sensitivity to the digestion with specific REs in distinct intraheterochromatic DNA subsets. In addn., these results show that enzymic prodn. of ss-DNA seems to be adequate for electron microscopy work since the

chromatin fiber is preserved better than in denatured DNA produced with heat, NaOH, or formamide.
IT 9037-44-9, Exonuclease III
RL: USES (Uses)
(chromosomes of L-929 mouse cells selective digestion with restriction endonucleases and, oligonucleotide priming of single-stranded DNA after, intraheterochromatic DNA subsets in relation to)

L9 ANSWER 5 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 5

TI Low incorporation of dUMP by some thermostable DNA polymerases may limit their use in PCR amplifications
AU Slupphaug, Geir; Alseth, Ingunn; Eftedal, Ingrid; Volde, Gunnar; Krokan, Hans E.
CS UNIGEN Cent. Mol. Biol., Univ. Trondheim, N-7005, Norway
SO Anal. Biochem. (1993), 211(1), 164-93
DT JOURNAL CODEN: ANBCA2; ISSN: 0003-2697

AB Incorporation of dUMP instead of dTMP is frequently used to control carryover contamination during PCR amplifications. Four thermostable DNA polymerases were tested for their ability to utilize dUTP as a substrate in PCR. Amplification of products in the presence of dUTP instead of dTTP was good with *Thermus aquaticus* DNA polymerase but highly inefficient with three other thermostable DNA polymerases. The latter was due to: (a) lower incorporation of dUMP relative to dTMP, (b) increased proofreading toward dUMP in DNA, (c) reactions in the presence of dUTP, and (d) thermostable dUTPase activity in the com. enzyme prepn. The last point only applies to *Pyrococcus furiosus* DNA polymerase. This study demonstrates that various thermostable DNA polymerases utilize dTTP and dUTP with highly different efficiencies and thus the choice of DNA polymerase may be crit. for amplification of DNA.

IT 1173-82-6, DUTP
RL: PROC (Process)
(differentiation utilization of, by thermostable DNA polymerase from *Thermus aquaticus* and *Thermococcus litoralis* and *Pyrococcus furiosus*, in PCR)

L9 ANSWER 6 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 6

TI Roles of uracil-DNA glycosylase and apyrimidinase endonucleases in the molecular 5-bromo-2'-deoxyuridine photosensitization
AU Yamamoto, Yoko; Fujiwara, Yoshisada
CS Sch. Med., Kobe Univ., Kobe, 650, Japan
SO Photochem. Photobiol. (1993), 58(1), 66-70
DT JOURNAL CODEN: PHCBAP; ISSN: 0031-8655

LA English

AB The mol. mechanism for 5-bromo-2'-deoxyuridine (BrdU) photosensitization was studied in thymine-requiring wild-type and uracil-DNA glycosylase (UDG)-deficient ung mutant cells of Escherichia coli K-12. Wild-type cells were more sensitive to BrdU photosensitization than ung mutant cells. UV-induced the identical nos. of alk. sucrose single-strand breaks (SSB) in 5-bromouracil-DNA (BrU-DNA) of both the wild-type and ung mutant. The ung mutant cells repaired SSB almost completely, whereas the wild-type cells with UDG produced more adverse SSB by 90 min after UV. Neutral agarose gel electrophoresis of minipreps indicated that UV induced (1) more smears of host BrU-DNA possibly by more double-strand breaks (DSB) and (2) a greater decline of pBR322 Form I BrU-DNA in the wild-type cells than the ung cells. These results indicated a greater induction of SSB by apyrimidinic (AP) endonucleases in wild-type cells. The ung/wild ratios (=1.7-1.9) for cellular and plasmid BrdU sensitizations after growth in 50% BrdU were similar. The extents of UDG-dependent and UDG-independent sensitizations in wild-type cells were .apprx.40 and .apprx.60%, resp. The xth nfo double mutant defective in both exonuclease III and endonuclease IV was more sensitive to BrdU photosensitization than the wild type, indicating that an excess of AP sites remaining after uracil excision in the xth nfo mutant causes a greater BrdU photosensitization than SSB by AP endonucleases in wild-type cells. Conversely, the xth nfo ung triple mutant was more resistant to BrdU photosensitization than the xth nfo double mutant, so that UV-induced uracil residues in the BrU-DNA are tolerated and do not appear to be directly responsible for BrdU photosensitization.

IT 9037-44-9, Exonuclease III

RL: BIOL (Biological study)

(in bromodeoxyuridine photosensitization of Escherichia coli)

L9 ANSWER 7 OF 15 CA COPYRIGHT 1996 ACS

DUPLICATE 7

AN 117:21509 CA

TI Sensitive detection process for nucleic acids

IN Kessler, Christoph; Rueger, Ruediger; Seibl, Rudolf; Kruse-Mueller, Cornelia; Berner, Sibylle

PA Boehringer Mannheim G.m.b.H., Germany

SO PCT Int. Appl., 53 pp.

CODEN: PIXXD2

PI WO 9206216 A1 920416

DS W: AU, BG, BR, CA, CS, FI, HU, JP, KR, NO, PL, RO, SU, US

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE

AI WO 91-EP1898 911004

PRAI DE 90-4032024 901009

DE 90-4038804 901205

DE 90-4041608 901222

DT Patent

LA German

AB A nucleic acid A is specifically detected in a sample by (1) reacting the sample with .gtoreq.1 labeled mononucleoside triphosphates, a primer, and .gtoreq.1 enzyme (polymerase) which catalyzes the formation of a labeled nucleic acid B, complementary to A, contg. this nucleotide, and nonthermally denaturing B; (2)

reacting the sample with a nucleic acid probe C, contg. an immobilizable group, which is adequately complementary to B; (3) contacting hybrid nucleic acid D, formed from hybridization of B and C, with a solid phase capable of binding C, sepg. the solid and liq. phases, and detecting the label bound to the solid phase. Thus, target hepatitis B virus DNA was amplified by PCR using *Thermus aquaticus* DNA polymerase and primers which bind to positions 1937-1960 and 2434-2460 in the presence of digoxigenin 11-(2'-deoxyuridine 5'-triphosphate), and the product, together with biotin-labeled cloned hepatitis B virus DNA, was denatured with 0.5M NaOH. This product was hybridized with a probe (not specified) in a microtiter well coated with streptavidin, and the bound complex was detected by incubation with (a) anti-digoxigenin antibody-peroxidase conjugate and (b) ABTS and measurement of the extinction at 405 nm.

L9 ANSWER 8 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 8
 AN 117:63880 CA
 TI 'Run-off' polymerization with digoxigenin labeled nucleotides creates highly sensitive and strand specific DNA hybridization probes: synthesis and application
 AU Stuerzl, Michael; Oskoul, Kaveh Bastani; Roth, Willi Kurt
 CS Dep. Virus Res., Max-Planck-Inst. Biochem., Martinsried, D-8033,
 Germany
 SO Mol. Cell. Probes (1992), 6(2), 107-14
 CODEN: MCPRE6; ISSN: 0890-8508
 DT Journal
 LA English
 AB In this paper the in vitro synthesis and application of

non-radioactively labeled strand specific DNA probes is described. The probe is labeled by incorporation of nucleotides with the hapten digoxigenin into single-stranded DNA during a 'run-off' reaction catalyzed by *Thermus aquaticus* (Taq) DNAQ-polymerase. The 'run-off' reaction requires a linearized plasmid template and one primer binding site at a defined distance from the restriction site. Single-stranded DNA can be synthesized during repeated cycles of denaturation, annealing, and extension. The conditions for the incorporation of digoxigenin-11-dUTP (dig-11-dUTP) during polymerization were optimized to generate strand specific DNA hybridization probes up to a length of 5000 nt. The strand specificity is demonstrated by a dot blot, with in vitro-transcribed target RNA of c-sis. The sensitivity of the probe was tested in a Northern blot, and found to be identical to a probe radiolabeled by nick-translation (specific activity 6.5 times. 108 cpm. μ g-1). The resolution of the signals and speed of development was even superior compared to the radiolabeled probe.

L9 ANSWER 9 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 9
 AN 114:180450 CA
 TI Formation of DNA triplexes accounts for arrests of DNA synthesis at
 AU Baran, Nava; Lapidot, Aviava; Manor, Haim
 CS Dep. Biol., Technion-Israel Inst. Technol., Haifa, 32000, Israel
 d(TC)n and d(GA)n tracts

SO Proc. Natl. Acad. Sci. U. S. A. (1991), 88(2), 507-11
CODEN: PNASA6; ISSN: 0027-8424

DT Journal

LA English

AB To study the mechanism of arrest of DNA synthesis at d(TC)_n and d(GA)_n sequences, single-stranded DNA mols. including d(TC)₂₇ or d(TC)₃₁ tracts or a d(GA)₂₇ tract were used as templates for in vitro assays of cDNA synthesis performed by extension of a primer with the Klenow polymerase or the Taq polymerase (*Thermus aquaticus* DNA polymerase).

Electrophoresis of the products revealed that arrests occurred around the middle of these tracts. The arrests in the d(TC)_n sequences were eliminated when dATP or dGTP was replaced with the analog 7-deaza dATP or 7-deaza dGTP, resp., or when the templates were preincubated with the *Escherichia coli* single-strand binding protein (SSB). Preincubation of the template including a d(GA)₂₇ tract with SSB has also eliminated the arrests at this sequence. Furthermore, arrests did not occur at d[G(7-deaza A)]₂₇ or d[(7-deaza G)A]₂₇ tracts when mols. including such tracts were used as templates. These results are compatible with the notion that the arrests were caused by formation of d(TC)_i.cntdot.d(GA)_i.cntdot.d(TC)_i and d(GA)_i.cntdot.d(GA)_i.cntdot.d(TC)_i triplexes, in which the bases in the uncopied portions of the d(TC)_n tracts, or of the d(GA)₂₇ tract, and the purine bases in the newly synthesized d(TC)_i.cntdot.d(GA)_i duplexes were bound by hydrogen bonds. In the assays performed with the Taq polymerase, the pH dependence (in the range of 6.0-9.0) and the temp. dependence of the arrests were detd. As the pH was lowered, the arrests in the d(TC)₂₇ tract were enhanced, in line with the expected properties of d(TC)_i.cntdot.d(GA)_i.cntdot.d(TC)_i triplexes. The arrests in the d(GA)₂₇ tract were enhanced by an increase in the pH. At pH 7.2 the arrests in the d(GA)₂₇ tract persisted up to 80.degree., whereas the arrests in the d(TC)₂₇ tract were eliminated at 50.degree.; these results presumably reflect the relative stabilities of the two triplexes mentioned above at this physiol. pH value and could be biol. significant.

L9 ANSWER 10 OF 15 CA COPYRIGHT 1996 ACS

DUPLICATE 10

AN 113:207798 CA

TI The use of *E. coli* exonuclease III to generate single stranded DNA in BrdUrd cell-cycle analysis permits simultaneous detection of cell surface antigens

AU Bayer, Jan A.; De Vries, Peter; Herweijer, Hans; Bauman, Jan G. J.

CS Inst. Radiobiol. Immunol., TNO, Rijswijk, Neth.

SO J. Immunol. Methods (1990), 132(1), 13-24

CODEN: JIMMBG; ISSN: 0022-1759

DT Journal

LA English

AB An immunocytochem. method for the simultaneous flow cytometric quantitation of total cellular DNA, incorporated 5-bromo-2'-deoxyuridine (BrdUrd) and one or more cell surface antigens has been developed. Biotin labeling of cell surface antigens,

critically tuned fixation techniques, and an enzymic denaturation of cellular DNA are the essential features of this method. Enzymic denaturation of cellular DNA prevented loss of cell surface antigen-bound biotin moieties, and thus preserved cell surface immunofluorescence distribution. After a mild protein extn. and the introduction of breaks into the chromatin using restriction endonucleases, *Escherichia coli* exonuclease III was used to generate stretches of single-stranded DNA. This approach permits detections of the incorporated BrdUrd using anti-BrdUrd monoclonal antibodies. The enzymic denaturation protocol was optimized using in vitro BrdUrd-labeled L1210 murine leukemia cells, and applied to both in vivo and ex vivo BrdUrd-labeled murine bone marrow cells. With this new method it is possible to study DNA content, cell cycle kinetics and cell surface antigen expression simultaneously, and hence functional relationships between these parameters can be investigated.

IT 9037-44-9, Exodeoxyribonuclease III
 RL: ANST (Analytical study)
 (cellular DNA denaturation by, for simultaneous detection of cell surface antigens and single-stranded DNA in cell cycle by immunofluorescence)

L9 ANSWER 11 OF 15 CA COPYRIGHT 1996 ACS DUPLICATE 11

AN 112:135591 CA
 TI Method for rapid base sequencing of DNA and RNA
 IN Jett, James Hubert; Keller, Richard Alan; Martin, John Calvin; Moyzis, Robert Keith; Shera, Edgar Brooks; Stewart, Carleton Colburn; Ratliff, Robert Lafayette
 PA United States Dept. of Energy, USA
 SO PCT Int. Appl., 23 pp.
 CODEN: PIXXD2

PI WO 8903432 A1 890420
 DS W: JP, NO
 AI RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE
 WO 88-US3194 880916
 PRAI US 87-105375 871007

DT Patent
 LA English
 AB

In the title method, a single fragment of DNA or RNA is provided with identifiable bases and suspended in a moving flow stream, an exonuclease sequentially cleaves individual bases from the end of the suspended fragment, and the moving flow stream maintains the cleaved bases in an orderly train for subsequent detection. Individual bases may be labeled with characteristic fluorescent dyes, and the train of bases excited to fluoresce with the output spectrum characteristic of the individual bases. The process may be automated and can process long (kilobases) strands of DNA or RNA. Poly(A,G)2138 was synthesized using a poly(T)7 primer in a terminal deoxyribonucleotidyl transferase-catalyzed reaction. The complementary strand was then synthesized with biotin-labeled dCTP and biotin-labeled dUTP. The biotinylated nucleic acid duplex was sequentially cleaved with exonuclease III at 37.degree. and pH 8. After 2 h, 30% of the DNA was cleaved, and the cleavage

reaction appeared to be still proceeding. Reaction with a nonbiotinylated control DNA yielded 85% cleavage in 2 h. Biotinylation appeared to slow the cleavage reaction using exonuclease III, but the tagged nucleotides were sequentially cleaved from the fragments.

IT 9037-44-9, Exonuclease III

RL: ANST (Analytical study)

(cleavage of biotinylated DNA with, DNA sequence anal. by exonuclease cleavage in flow stream in relation to)

L9 ANSWER 12 OF 15 CA COPYRIGHT 1996 ACS

DUPLICATE 12

AN 110:89890 CA

TI DNA sequencing with *Thermus aquaticus* DNA
polymerase and direct sequencing of polymerase chain
reaction-amplified DNA

AU Innis, Michael A.; Myambo, Kenneth B.; Gelfand, David H.; Brow, Mary
Ann D.

CS Dep. Microb. Genet., Cetus Corp., Emeryville, CA, 94608, USA

SO Proc. Natl. Acad. Sci. U. S. A. (1988), 85(24), 9436-40

CODEN: PNASA6; ISSN: 0027-8424

DT Journal

LA English

AB The highly thermostable DNA polymerase from *T. aquaticus* (Taq) is ideal for both manual and automated DNA sequencing because it is fast, highly processive, has little or no 3'-exonuclease activity, and is active over a broad range of temps. Sequencing protocols are presented that produce readable extension products >1000 bases having uniform band intensities. A combination of high reaction temps. and the base analog 7-deaza-2'-deoxyguanosine was used to sequence through G + C-rich DNA and to resolve gel compressions. The polymerase chain reaction (PCR) conditions for direct DNA sequencing of asym. PCR products without intermediate purifn. were modified by using Taq DNA polymerase. The coupling of template prepn. by asym. PCR and direct sequencing should facilitate automation for large-scale sequencing projects.

L9 ANSWER 13 OF 15 CA COPYRIGHT 1996 ACS

DUPLICATE 13

AN 100:188438 CA

TI Modified labeled nucleotides and polynucleotides and methods of
utilizing and detecting them

IN Engelhardt, Dean; Rabbani, Elazar; Kline, Stanley; Stavrianopoulos,
Jannis G.; Kirtikar, Dollie

PA Enzo Biochem, Inc., USA

SO Eur. Pat. Appl., 140 pp.

CODEN: EPXXDW

PI EP 97373 A2 840104

DS R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

AI EP 83-106112 830622

PRAI US 82-391440 820623

DT Patent

LA English

AB Nucleotides, polynucleotides, and DNA were chem. modified or labeled
with chem. moieties which were readily detectable. These chem.

moieties included carbohydrates and sugars, electron dense substances, magnetic substances, enzymes, coenzymes, hormones, radioactive substances, metals, fluorescent substances, antigens, or antibodies. These chem. modified nucleotides were used for: (1) stimulating or inducing cells to produce lymphokines, cytokines, and interferon; (2) testing resistance of bacteria to antibiotics; (3) diagnosing genetic disorders, e.g., beta-thalassemia; (4) diagnosing tumors; (5) diagnosing bacteria, virus, or fungus infection; and (6) karyotyping chromosomes.

IT 9037-44-9

RL: ANST (Analytical study)

(digested by, of polydeoxyadenylic acid-polythymidylic acid)

L9

ANSWER 14 OF 15 CA COPYRIGHT 1996 ACS

DUPLICATE 14

AN

97:106747 CA

TI

Role of exonuclease III in the base excision repair of

AU

uracil-containing DNA

Taylor, Andrew F.; Weiss, Bernard
Sch. Med., Johns Hopkins Univ., Baltimore, MD, 21205, USA

SO

J. Bacteriol. (1982), 151(1), 351-7

DT

Journal

LA

English

AB

Mutants of *Escherichia coli* K-12 deficient in both exonuclease III

(the product of the xth gene) and deoxyuridine triphosphatase (the dut gene product) are inviable at high temps. and undergo filamentation when grown at such temps. In dut mutants, the dUTP pool is greatly enhanced, resulting in an increased substitution of uracil for thymine in DNA during replication. The subsequent removal of uracil from the DNA by uracil-DNA glycosylase produces apyrimidinic sites, at which exonuclease III has an endonucleolytic activity. The lethality of dut xth mutants, therefore, indicates that exonuclease III is important for this base-excision pathway and suggests that unrepaired apyrimidinic sites are lethal. The dut xth mutants were viable if they also had a mutation in the uracil-DNA glycosylase (ung) gene; such mutants should not remove uracil from DNA and should not, therefore, generate apyrimidinic sites. In the majority of the temp.-resistant revertants isolated, viability had been restored by a mutation in the dCTP deaminase (dcd) gene; such mutations should decrease dUTP prodn. and hence uracil misincorporation. Apparently, in dut mutants, exonuclease III is essential for the repair of uracil-contg. DNA and of apyrimidinic sites.

IT 9037-44-9

RL: BIOL (Biological study)

(of *Escherichia coli*, in base excision repair of uracil-contg. DNA)

L9

ANSWER 15 OF 15 CA COPYRIGHT 1996 ACS

DUPLICATE 15

AN

91:153410 CA

TI

DNA proof-reading by a eukaryotic DNA polymerase

AU

Yarranton, G. T.; Banks, G. R.

CS Natl. Inst. Med. Res., London, NW7 1AA, Engl.
SO NATO Adv. Study Inst. Ser., Ser. A (1978), Volume Date 1977, A17(DNA
Synth.: Present Future), 479-86
CODEN: NASSDK; ISSN: 0161-0449

DT Journal

LA English

AB The 3' .fwdarw. 5'-exonuclease assocd. with Ustilago maydis DNA polymerase hydrolyzed the noncomplementary dAMP-3H termini in poly(dA).cntdot.(dT)320-dA0.97-3H (I) .apprx.4-fold faster than complementary dTMP-3H in poly(dA).cntdot.(dT)320-dT1.7-3H (II) at 37.degree.; hydrolysis was 12-fold faster at 25.degree.. Similar results were obtained for the hydrolysis of noncomplementary dCMP-3H in poly(dA).cntdot.(dT)320-dC0.82-3H. Thus, the exonuclease shows a preference for noncomplementary 3'-terminal nucleotides which is enhanced at low temps. When II was incubated with enzyme in the presence of dTTP-.alpha.-32P, poly(dT)-32P synthesis was obsd. accompanied by an initial hydrolysis of .apprx.10% of the dTMP-3H, the remainder being resistant. This resistance required substrates (dTTP or dUTP) complementary to the template, suggesting a requirement for primer extension by polymerase. However, complete and rapid hydrolysis of dAMP-3H termini in I occurred as poly(dT)-32P synthesis proceeded. Thus, when extension of a complementary 3'-terminus occurs, it is apparently blocked from hydrolysis, whereas extension of a noncomplementary terminus is blocked until complementarity is restored by the exonuclease. The exonuclease is apparently specific for the double-stranded conformation and may bind to double-stranded DNA at a 3'-terminus on 1 strand with the exonuclease site only accommodating 1-3 single-stranded nucleotides. It was concluded that U. maydis DNA polymerase can proof-read in vitro, with the assocd. exonuclease removing noncomplementary primer nucleotides, thus restoring complementarity and providing functional primer termini for the polymerase activity.

IT 71631-66-8

RL: BIOL (Biological study)

(DNA polymerase-assocd., of Ustilago maydis, DNA proofreading by)

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L20 15 DUP REM L19 (14 DUPLICATES REMOVED)

=> d 1-15 bib abs; fill ca, caplus; tag polymerase# and (15 or 7(w)deaza? or
dudp or d utp or deoxyuridinetriphosphate or deoxyuridine? or deoxy
uridine?)

L20 ANSWER 1 OF 15 BIOTCHDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN 94-11411 BIOTCHDS
TI Single-buffer combined reverse transcription and DNA amplification
by a Thermus thermophilus DNA-
polymerase;
thermostable DNA-polymerase with reverse-transcriptase activity
application in simultaneous polymerase chain reaction and
reverse transcription (conference abstract)
Siga C L; Gelfand D H; Myers T W
Roche-Mol.Syst.
Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501,
USA.

SO Abstr.Gen.Meet.Am.Soc.Microbiol.; (1994) 94 Meet., 551
CODEN: 0005P

DT Journal
 LA English
 AN 94-11411 BIOTECHDS
 AB A recombinant DNA-polymerase (EC-2.7.7.7) from *Thermus thermophilus* (rTth pol) possesses efficient reverse-transcriptase (EC-2.7.7.49) activity in the presence of Mn²⁺. This may facilitate use of a single enzyme for reverse transcription (RT) and polymerase chain reaction (PCR), allow for increased primer binding specificity and alleviate the secondary structures present in the RNA template. Reaction conditions compatible with rTth pol performing both RT and PCR in a buffer containing Mn²⁺ were determined. The Mn²⁺ concentration optimum was different for RNA and DNA templates and the reaction was very sensitive to the free Mn²⁺ concentration unless a metal buffer such as bicine was used. Detection of a specific mRNA from 80 pg of total cellular RNA or 100 copies of synthetic cRNA was possible by standard analysis. The speed, sensitivity, robustness, and the ability to incorporate **dUTP** for carryover prevention by uracil-N-glycosylase achieved by using rTth pol for RT/PCR further extended their ability to detect cellular and viral RNA. Coupling of this assay with a quantitative detection system would make disease monitoring feasible. (0 ref)

L20 ANSWER 2 OF 15 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
 AN 95139437 EMBASE
 TI Sister chromatid differentiation after in situ detection of ultraviolet-induced DNA breaks under electron microscopy.
 AU Fernandez J.L.; Campos A.; Goyanes V.; Buno I.; Gosalvez J.
 CS Centro Oncologico de Galicia, Lab Dosimetria Biologica-Genetica, Avda de Montserrat s/n, 15006 La Coruna, Spain
 SO Biology of the Cell, (1994) 82/1 (33-37).
 ISSN: 0248-4900 CODEN: BCELDF
 CY France
 DT Journal
 FS 022 Human Genetics
 029 Clinical Biochemistry
 LA English
 SL English
 AB Chinese hamster DON cells with 5-bromodeoxyuridine (BrdU)-substituted chromosomes were ultraviolet (UV)-exposed and processed for in situ detection of induced DNA breaks under electron microscopy. For this purpose, UV-induced breaks were amplified by an exonuclease III digestion to obtain single stranded DNA motifs which could hybridize with oligonucleotides of random sequences. These reannealed motifs could be used as primers which were extended by the Klenow polymerase, incorporating biotinylated-**dUTP** that was detected by a gold-tagged streptavidin. After processing, the chromatid whose DNA was BrdU-substituted in one strand showed a higher electron density than the chromatid substituted in both strands. In contrast, the unifilarly substituted chromatid showed about twice the labelling of DNA breaks as the bifilarly substituted one. This result could be the consequence of a greater loss of chromatin tracts in the bifilarly substituted chromatid, as implied

by an X-ray microanalysis which showed that the amount of phosphorous lost by the bifilarly substituted chromatin was higher than that of the unifilarly substituted chromatin.

L20 ANSWER 3 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS

AN 94:60901 BIOSIS

DN 97073901

TI Selective digestion of mouse chromosomes with restriction endonucleases: oligonucleotide priming of single-stranded DNA produced with exonuclease III.

AU Gosalvez J, Lopez-Fernandez C, De La Vega C, Mezzanotte R, Fernandez J, Goyanes V

CS Dep. Biol. Edificio de Biol., Fac. de Ciencias, Universidad Autonoma de Madrid, 28049 Madrid, SPA

SO Genome 36 (2). 1993. 230-234. ISSN: 0831-2796

LA English

AB

L-929 mouse chromosomes prepared for electron microscopy have been treated with MspI, EcoRI, and HaeIII restriction endonucleases (REs). RE-induced nicks were amplified with exonuclease III to obtain single-stranded DNA (ss-DNA) motifs. The ss-DNA produced was enough to permit hybridization of a series of random oligonucleotides. These can be used as primers, which are extended by the Klenow fragment using non-isotopic labeled dUTP. The incorporation of biotinylated dUTP is detected with a gold-tagged streptavidin as the reporter molecule. This allows, in mouse chromosomes, the detection of different rates of sensitivity to the digestion with specific REs in distinct intranuclear DNA subsets. In addition, these results show that enzymatic production of ss-DNA seems to be adequate for electron microscopy work since the chromatin fiber is preserved better than in denatured DNA produced with heat, NaOH, or formamide.

L20 ANSWER 4 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS

AN 93:317417 BIOSIS

DN BA96:25767

TI DETECTION OF DNA STRAND BREAKS INDUCED BY HYDROXYL RADICALS IN

AU FERNANDEZ J L, GOSALVEZ J, GOYANES V J

CS LAB. DOSIMETRIA BIOL., CENT. ONCOL. DE GALICIA, AVDA DE MONTESERRAT S/N, 150006 LA CORUNA, SPAIN.

SO CYTOBIOS 73 (294-295). 1993. 189-195. CODEN: CYTBAI ISSN: 0011-4529

LA English

AB

Chinese hamster Don cells were treated with 10 mM hydrogen peroxide. DNA strand breaks induced by hydroxyl radicals were amplified in 3' termini by an exonuclease III digestion, resulting in single stranded DNA motifs. In situ detection of these motifs was performed on chromatin fibres of isolated whole-mounted nuclei and chromosomes by a random priming procedure, using biotinylated-dUTP which bound a gold-tagged streptavidin. This approach facilitated the location of hydroxyl radical induced DNA breaks, specifically on 20-30 nm diameter chromatin fibres, by transmission electron microscopy.

L20 ANSWER 5 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 1

AN 93:319748 BIOSIS

DN BA96:28098

TI LOW INCORPORATION OF DUMP BY SOME THERMOSTABLE DNA POLYMERASES MAY LIMIT THEIR USE IN PCR AMPLIFICATIONS.

AU SLUPPHAUG G; ALSETH I; EFTEDAL I; VOLDEN G; KROKAN H E

CS UNIGEN CENT. MOL. BIOL., UNIV. TRONDHEIM, N-7005 TRONDHEIM, NORWAY.

SO ANAL BIOCHEM 211 (1). 1993. 164-169. CODEN: ANBCA2 ISSN: 0003-2697

LA English

AB Incorporation of dUMP instead of dTMP is frequently used to control carryover contamination during PCR amplifications. We have tested four thermostable DNA polymerases for their ability to utilize **dUTP** as a substrate in PCR. Amplification of products in the presence of **dUTP** instead of dTTP was good with *Thermus aquaticus* DNA polymerase but highly inefficient with three other thermostable DNA polymerases. The latter was due to: (a) lower incorporation of dUMP relative to dTMP, (b) increased proofreading toward dUMP in DNA, (c) relative termination at dUMP residues as verified by sequencing reactions in the presence of **dUTP**, (d) thermostable dUTPase activity in the commercial enzyme preparation. The last point only applies to *Pyrococcus furiosus* DNA polymerase. This study demonstrates that various thermostable DNA polymerases utilize dTTP and **dUTP** with highly different efficiencies and thus the choice of DNA polymerase may be critical for amplification of DNA.

L20 ANSWER 6 OF 15 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.

AN 92211618 EMBASE

TI Characterization of biotinylated repair regions in reversibly permeabilized human fibroblasts.

AU Huijzer J.C.; Smerdon M.J.

CS Dept. of Biochemistry and Biophysics, Washington State University, Pullman, WA 99164-4660, United States

SO BIOCHEMISTRY, (1992) 31/21 (5077-5084).

ISSN: 0006-2960 CODEN: BICHAW

CY United States

DT Journal

FS 029 Clinical Biochemistry

LA English

SL English

AB We have examined the incorporation of biotinyl-11-deoxyuridine triphosphate (BiodUTP) into excision repair patches of UV-irradiated confluent human fibroblasts. Cells were reversibly permeabilized to BiodUTP with lysolecithin, and biotin was detected in DNA on nylon filters using a streptavidin/alkaline phosphatase colorimetric assay. Following a UV dose of 12 J/m², maximum incorporation of BiodUTP occurred at a lysolecithin concentration (80-100 .mu.g/mL) similar to that for incorporation of dTTP. Incorporation of BiodUTP into repair patches increased with UV dose up to 4 and 8 J/m² in two normal human fibroblast strains, while no incorporation of BiodUTP was observed in xeroderma pigmentosum (group A) human fibroblasts. The repair-incorporated biotin was not removed from the DNA over a 48-h period, and only

slowly disappeared after longer times (.apprx.30% in 72 h), while little of the biotin remained in cells induced to divide. Furthermore, the stability of the biotin in repaired DNA was unaffected by a second dose of UV radiation several hours after the biotin-labeling period to induce a 'second round' of excision repair. Exonuclease III digestion and gap-filling with DNA polymerase I indicate that the majority of biotin-labeled repair patches (.apprx.80%) are rapidly ligated in confluent human cells. However, the remaining patches were not ligated after a 24-h chase period, in contrast to dTTP-labeled repair patches. The Bioummp repair label in both chromatin and DNA is preferentially digested by staphylococcal nuclease, preventing the use of this enzyme for nucleosome mapping in these regions. However, restriction enzyme and DNase I digestions of the isolated nuclei demonstrate that at least some of the repair-incorporated Bioummp becomes associated with nucleosome core DNA following nucleosome rearrangement. Therefore, the biotin tag does not appear to prevent the folding of nascent repair patches into native nucleosome structures.

L20 ANSWER 7 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2

AN 91:158854 BIOSIS

DN BA91:84654

TI FORMATION OF DNA TRIPLEXES ACCOUNTS FOR ARRESTS OF DNA SYNTHESIS AT

DTC-N AND DGA-N TRACTS.

AU BARAN N; LAPIDOT A; MANOR H

CS DEP. BIOL., TECHNION-ISRRAEL INST. TECHNOL., HAIFA 32000, ISRRAEL.

SO PROC NATL ACAD SCI U S A 88 (2). 1991. 507-511. CODEN: PNASA6 ISSN:

0027-8424

LA English

AB

To study the mechanism of arrest of DNA synthesis at d(TC)n and d(GA)n sequences, single-stranded DNA molecules including d(TC)27 or d(TC)31 tracts or a d(GA)27 tract were used as templates for in vitro assays of complementary DNA synthesis performed by extension of a primer with the Klenow polymerase or the Taq polymerase (Thermus aquaticus DNA polymerase). Electrophoresis of the products revealed that arrests occurred around the middle of these tracts. The arrests in the d(TC)n sequences were eliminated when dATP or dGTP was replaced with the analogue 7-deaza dATP or 7-deaza dGTP, or when the templates were preincubated with the Escherichia coli single-strand binding protein (SSB). Preincubation of the template including a d(GA)27 tract with SSB has also eliminated the arrests at this sequence. Furthermore, arrests did not occur at d[G(7-deaza A)]27 or d[(7-

deaza G)A]27 tracts when molecules including such tracts were used as templates. These results are compatible with the notion that the arrests were caused by formation of d(TC)1 .cntdot. d(GA)1 .cntdot. d(TC)1 triplexes, in which the bases in the uncopied portions of the d(TC)n tracts, or of the d(GA)27 tract, and the purine bases in the newly synthesized d(TC)1 .cntdot. d(GA)1 duplexes were bound by hydrogen bonds. In the assays performed with the Taq polymerase, the pH dependence (in the range of 6.0-9.0) and the temperature dependence of the arrests were

determined. As the pH was lowered, the arrests in the d(TC)27 tract were enhanced, in line with the expected properties of d(TC)i .cntdot. d(GA)i .cntdot. d(TC)i triplexes. The arrests in the d(GA)27 tract were enhanced by an increase in the pH. At pH 7.2 the arrests in the d(GA)27 tract persisted up to 80.degree. C, whereas the arrests in the d(TC)27 tract were eliminated at 50.degree. C; these results presumably reflect the relative stabilities of the two triplexes mentioned above at this physiological pH value and could be biologically significant.

L20 ANSWER 8 OF 15 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 90-08045 BIOTECHDS
TI Structure-independent DNA amplification;
using polymerase chain reaction with incorporation of 7
-deaza-2'-deoxyguanosine-5'-triphosphate
PA Cetus
PI WO 9003443 5 Apr 1990
AI WO 89-US4100 19 Sep 1989
PRAI US 88-248556 23 Sep 1988
DT Patent
LA English
OS WPI: 90-132279 [17]
AN 90-08045 BIOTECHDS
AB A method for structure-independent DNA amplification using the polymerase chain reaction (PCR) is new. It comprises: (1) hybridizing DNA with a pair of oligonucleotide primers, an agent for polymerization, dATP, dCTP, TTP and c7dGTP to form an extension product, which can function as a template; (2) separating the extension product from the templates; and (3) repeating steps (1) and (2) using the extension product formed in step (2). The dGTP is also present in step (1) and the agent for polymerization is preferably a thermostable DNA-polymerase (EC-2.7.7.7), especially *Thermus aquaticus* DNA-polymerase.
The DNA is prepared from RNA by synthesizing cDNA from RNA and making the cDNA double stranded. The concentration of dATP, TTP, dCTP, dGTP and c7dGTP is preferably 10 uM-1.5 mM. The ratio of dGTP and c7dGTP is preferably 1:3 and the dGTP is preferably used at 50 uM. The c7dGTP is preferably incorporated in cDNA and is double-stranded. The incorporation of 7-deazaguanine into the amplified DNA results in an increase in specificity of PCR on templates that contain secondary structure and/or compressed regions. (16pp)

L20 ANSWER 9 OF 15 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 90-132278 [17] WPIDS
DNC C90-058134
TI Nucleotide sequence determ. by chain termination - using DNA polymerase from *Thermus aquaticus* to catalyse primer extension reactions.
DC B04 D16
IN BROW, M A D; GELFAND, D H; INNIS, M A; MYAMBO, K B; BROW, M A
PA (HOFF) HOFFMANN LA ROCHE & CO AG F; (CETU) CETUS CORP; (HOFF) HOFFMANN-LA ROCHE AG F

CYC 15

PI WO 9003442 A 900405 (9017)*

RW: AT BE CH DE FR GB IT LU NL SE

W: AU JP

AU 8943003 A 900418 (9027)

EP 437459 A 910724 (9130)

R: AT BE CH DE FR GB IT LU NL SE

US 5075216 A 911224 (9203)

JP 04501205 W 920305 (9216)

AU 647015 B 940317 (9416)

CA 1332561 C 941018 (9442)

ADT EP 437459 A EP 89-910787 890919; US 5075216 A US 88-249367 880923; JP 04501205 W JP 89-510171 890919; AU 647015 B AU 89-43003 890919; CA 1332561 C CA 89-612500 890922

FDT JP 04501205 W Based on WO 903442; AU 647015 B Previous Publ. AU 8943003, Based on WO 9003442

PRAI US 88-249367 880923

AN 90-132278 [17] WPIDS

AB WO 9003442 A UPAB: 940223

A method for determining a nucleotide sequence for a nucleic acid segment by a dideoxynucleotide-5'-triphosphate chain termination procedure is claimed in which the sequence is detd. by extending an oligonucleotide primer in a template-dependent manner in the presence of *Thermus aquaticus* (Taq) DNA polymerase, 4 deoxyribonucleotide-5'-triphosphates (dNTPs) and a dideoxynucleotide-5'-triphosphate (ddNTP).

ADVANTAGE - The ability of Taq DNA polymerase to operate at high temp. and low salt allows heat-destabilisation of hairpins during the sequencing reaction, permitting the enzyme to read through such structures. The concomitant use of a structure-destabilising dGTP analogue such as 7-deaza-2'-deoxyguanosine-5'-triphosphate (c7dGTP) yields sequencing prods. from such difficult to sequence DNA that were fully resolved upon electrophoresis. The method provides an absence of background bands and uniform intensity of the radioactive fragments. The Taq DNA polymerase is very processive and has very little if any proofreading activity. @ (30pp DWg.No.0/3)

ABEQ US 5075216 A UPAB: 930928

Determin. of a nucleotide sequence in a nucleic acid segment by a dideoxynucleotide-5'-triphosphate chain termination method comprises extending an oligonucleotide primer according to a template, in the presence of *Thermus aquaticus* DNA polymerase, four deoxynucleoside-5'-triphosphate (ddATP, dGCTP, dGCTP or dGCTP); and a dideoxynucleoside-5'-triphosphate (ddATP, dGCTP, dGCTP or dGCTP); and electrophoretic analysis of the prods..

USE - The process is applicable to molecular biology, genetics, diagnosis and forensic science.

TI THE USE OF ESCHERICHIA-COLI EXONUCLEASE
 III TO GENERATE SINGLE STRANDED DNA IN BRDURD CELL-CYCLE
 ANALYSIS PERMITS SIMULTANEOUS DETECTION OF CELL SURFACE ANTIGENS.
 AU BAYER J A; DE VRIES P; HERWEIJER H; BAUMAN J G J
 CS TNO INST. APPLIED RADIOBIOL. IMMUNOL., DEP. CELL BIOL., CYTOMETRY
 SECT., P.O. BOX 5815, 2280 HV RIJSWIJK, NETHERLANDS.
 SO J IMMUNOL METHODS 132 (1). 1990. 13-24. CODEN: JIMMBG ISSN:
 0022-1759
 LA English
 AB An immunocytochemical method for the simultaneous flow cytometric
 quantitation of total cellular DNA, incorporated 5-bromo-2'-
deoxyuridine (.BETA.rdUrd) and one or more cell surface
 antigens has been developed. Biotin labeling of cell surface
 antigens, critically tuned fixation techniques and an enzymatic
 denaturation of cellular DNA are the essential features of this
 method. Enzymatic denaturation of cellular DNA was shown to prevent
 loss of cell surface antigen-bound biotin moieties, and thus to
 preserve cell surface immunofluorescence distribution. After a mild
 protein extraction and the introduction of breaks into the chromatin
 using restriction endonucleases, *E. coli*
exonuclease III was used to generate stretches of
 single stranded DNA. This approach permits detection of the
 incorporated BrdUrd using anti-BrdUrd monoclonal antibodies. The
 enzymatic denaturation protocol was optimized using in vitro
 BrdUrd-labeled L1210 murine leukemia cells, and applied to both in
 vivo and ex vivo BrdUrd-labeled murine bone marrow cells. With this
 new method it is possible to study DNA content, cell cycle kinetics
 and cell surface antigen expression simultaneously, and hence
 functional relationships between these parameters can be
 investigated.

L20 ANSWER 11 OF 15 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
 AN 89-01756 BIOTECHDS
 TI DNA sequencing using Taq polymerase;
 new method with *Thermus aquaticus* DNA-
polymerase
 AU Peterson M G
 LO The Walter and Eliza Hall Institute of Medical Research, PO Royal
 Melbourne Hospital, Victoria 3050, Australia.
 SO Nucleic Acids Res.; (1988) 16, 22, 10915
 CODEN: NARHAD
 DT Journal
 LA English
 AN 89-01756 BIOTECHDS
 AB A new method was developed using thermostable DNA-polymerase
 (EC-2.7.7.7) of *Thermus aquaticus* (Taq) in DNA sequencing
 incorporating **7-deaza**-GTP at 70 deg,
 eliminating inhibitory effects of DNA template secondary structure.
 For the labeling reaction, phage M13 primer was annealed to the
 phage M13 template and extended in the presence of labeled dNTPs.
 For the termination reaction, the product was divided into aliquots
 with appropriate deoxy/dideoxynucleotide mixes. The
 single-stranded phage M13 template used to test this method was a

cDNA fragment encoding lichenase (EC-3.2.1.73) from wheat (*Triticum aestivum*), containing a region with strong secondary structure that could not be unambiguously sequenced on either strand using sequenase. With sequenase at 37 deg, strong stops occurred in all 4 tracks at 1 position. Sequencing the template with Tag DNA-polymerase at 70 deg gave an unambiguous reading. Tag DNA-polymerase has further advantages over sequenase: it does not require a separate annealing reaction, is more stable, allowing all labeling constituents (except the template) to be mixed before aliquoting, and is much cheaper. (1 ref)

L20 ANSWER 12 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 5

DN 89:123948 BIOSIS

BA87:58601

TI

DNA SEQUENCING WITH THERMUS-AQUATICUS DNA

POLYMERASE AND DIRECT SEQUENCING OF POLYMERASE CHAIN

REACTION-AMPLIFIED DNA.

INNIS M A; MYAMBO K B; GELFAND D H; BROW M A D

DEP. MICROBIAL GENETICS, CETUS CORP., 1400 FIFTY-THIRD ST.,

EMERYVILLE, CA 94608.

SO PROC NATL ACAD SCI U S A 85 (24) . 1988. 9436-9440. CODEN: PNAS46

ISSN: 0027-8424

LA

English

AB

The highly thermostable DNA polymerase from *Thermus aquaticus* (Tag)

is ideal for both manual and automated DNA sequencing because it is

fast, highly processive, has little or no 3'-exonuclease activity,

and is active over a broad range of temperatures. Sequencing

protocols are presented that produce readable extension products >

1000 bases having uniform band intensities. A combination of high

reaction temperatures and the base analog 7-deaza

-2'-deoxyguanosine was used to sequence through G+C-rich DNA and to

resolve gel compressions. We modified the polymerase chain reaction

(PCR) conditions for direct DNA sequencing of asymmetric PCR products

without intermediate purification by using Tag DNA polymerase. The

coupling of template preparation by asymmetric PCR and direct

sequencing should facilitate automation for large-scale sequencing

projects.

L20

ANSWER 13 OF 15 BIOTECCHDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN

88-11096 BIOTECCHDS

TI

Rapid production of vector-free biotinylated probes using the

polymerase chain reaction;

construction of amplified biotin-labeled DNA probe

AU

Lo Y M D; Mehal W Z; Fleming K A

University of Oxford, Nuffield Department of Pathology, John

Radcliffe Hospital, Oxford, OX3 9DU, UK.

SO

Nucleic Acids Res.; (1988) 16, 17, 8719

CODEN: NARHAD

DT

Journal

LA

English

AN

88-11096 BIOTECCHDS

AB

Biotin-labeled vector-free DNA probes were produced by a polymerase chain reaction (PCR), with 150 mM dTTP and 50 uM biotinylated

dUTP (bio-dUTP). The target was 1 ng plasmid pHBV130 with a full length hepatitis-B virus insert. 5' And 3' primers were obtained from known sequences. 25 Cycles (2 min at 94 deg and 55 deg, 3 min at 72) were carried out. When the labeled product was used as a hybridization probe, 1 pg target DNA could be detected. As an alternative method, after amplification without bio-dUTP the mixture was diluted, denatured and 10-50 uM bio-dUTP was added, with labeling at 24, 37, 55 or 72 deg with or without additional **Thermus aquaticus DNA-polymerase (EC-2.7.7.7).** However, the first protocol was faster and more economical. To avoid non-specific labeling the amount of plasmid DNA was kept to 1 ng or below. The method was very specific and there was little hybridization to the vector band. 5-10 ug probe was synthesized in less than 4 hr. The method could be used generally for target sequences below 2.5 kb. A probe for detection of amplified product could be produced using primers internal to the target sequence. (4 ref)

L20 ANSWER 14 OF 15 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
 AN 89-03099 BIOTECHDS
 TI DNA sequencing with **Thermus aquaticus DNA-**

polymerase and direct sequencing of polymerase chain reaction-amplified DNA;
 DNA amplification

AU Innis M A; Myambo K B; Gelfand D H; Brow M A D
 CS Cetus
 LO Department of Microbial Genetics, Cetus Corporation, 1400
 Fifty-Third Street, Emeryville, CA 94608, USA.
 SO Proc.Natl.Acad.Sci.U.S.A.; (1988) 85, 24, 2436-40
 CODEN: PNASA6

DT Journal
 LA English

AN 89-03099 BIOTECHDS

AB The highly thermostable DNA-polymerase (EC-2.7.7.7) from *Thermus aquaticus* (Taq) is suitable for manual and automated DNA sequencing because it is rapid, has little or no 3'-exonuclease activity, and is active over a broad temp. range. DNA sequencing protocols were developed to produce readable extension products of over 1000 bases, having uniform band intensities. A combination of high reaction temp. and the base analog **7-deaza** -2'-deoxyguanosine was used to sequence G+C-rich DNA and to resolve gel compressions. The enzyme worked equally well with either 5'-labeled primers or by incorporation of label in a 2-step reaction protocol. Both approaches generated sequencing ladders free of background bands which were uniform and readable over long distances. The polymerase chain reaction conditions were modified for direct DNA sequencing of asymmetric polymerase chain reaction products without intermediate purification by using Taq DNA-polymerase. The coupling of template preparation by asymmetric polymerase chain reaction and direct sequencing should facilitate automation for large-scale DNA sequencing projects. (19 ref)

L20 ANSWER 15 OF 15 BIOSIS COPYRIGHT 1996 BIOSIS

AN 83:191449 BIOSIS
 DN BA75:41449
 TI ROLE OF EXO NUCLEASE III IN THE BASE EXCISION REPAIR OF URACIL
 CONTAINING DNA.
 AU TAYLOR A F; WEISS B
 CS DEP. MOLECULAR BIOL. AND GENETICS, JOHN HOPKINS UNIV. SCH. MED.,
 BALTIMORE, MD 21205.
 SO J BACTERIOL 151 (1). 1982. 351-357. CODEN: JOBAAY ISSN: 0021-9193
 LA English
 AB Mutants of Escherichia coli K-12 deficient in both exonuclease III

(the product of the xth gene) and deoxyuridine triphosphatase (the dtp gene product) are invariable at high temperatures and undergo filamentation when grown at such temperatures. In dut mutants, the dtp pool is known to be greatly enhanced, resulting in an increased substitution of uracil for thymine in DNA during replication. The subsequent removal of uracil from the DNA by uracil-DNA glycosylase produces apyrimidinic sites, at which exonuclease III is known to have an endonucleolytic activity. The lethality of dut xth mutants, therefore, indicates that exonuclease III is important for this base-excision pathway and suggests that unrepaired apyrimidinic sites are lethal. dut xth mutants were viable if they also had a mutation in the uracil-DNA glycosylase (ung) gene; such mutants should not remove uracil from DNA and should not, therefore, generate apyrimidinic sites. In the majority of the temperature-resistant revertants isolated, viability had been restored by a mutation in the dctp deaminase (dcd) gene; such mutations should decrease dtp production and hence uracil misincorporation. In dut mutants, exonuclease III is essential for the repair of uracil-containing DNA and of apyrimidinic sites.

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 For a list of commands available to you in the current file, enter
 "HELP COMMANDS" at an arrow prompt (=>).

=> s tag polymerase# and (15 or 7(w)deaza? or dtp or d utp or
 deoxyuridine triphosphate or deoxyuridine? or deoxy uridine?)
 L21 16 FILE CA
 L22 16 FILE CAPLUS

TOTAL FOR ALL FILES
 L23 32 TAG POLYMERASE# AND (15 OR 7(W) DEAZA? OR DTP OR D UTP OR

DEOXYURIDINETRIPHOSPHATE OR DEOXYURIDINE? OR DEOXY URIDIN

E?)

=> s 123 not 19

L#S CREATED BY SORT OR DUPLICATE ARE NOT ALLOWED

An L# created by a SORT or a DUPLICATE command cannot be used with the SEARCH command. Enter "DISPLAY HISTORY" at the arrow prompt (=>) to determine the original L#.

=> s 123 not 18

L24 14 FILE CA

L25 14 FILE CAPLUS

TOTAL FOR ALL FILES

L26 28 L23 NOT L8

=> dup rem 126

PROCESSING COMPLETED FOR L26

L36 14 DUP REM L26 (14 DUPLICATES REMOVED)

=> d his 121-126; d 136 1-14 .bevstr; fil

biosi,medl,embas,lifesci,biotechds,wpids,confsci,dissabs,scisearch

(FILE 'CA, CAPLUS' ENTERED AT 16:48:32 ON 08 MAY 96)

L21 16 FILE CA

L22 16 FILE CAPLUS

TOTAL FOR ALL FILES

L23 32 S TAQ POLYMERASE# AND (L5 OR 7(W)DEAZA? OR DUTP OR D UTP

L24 14 FILE CA

L25 14 FILE CAPLUS

TOTAL FOR ALL FILES

L26 28 S L23 NOT L8

L36 ANSWER 1 OF 14 CA COPYRIGHT 1996 ACS

DUPLICATE 1

AN 122:124149 CA

TI a more reliable PCR for detection of Mycobacterium tuberculosis in clinical samples

AU Kox, L. F. F.; Rhienthong, D.; Miranda, A. Medo; Udomsantisuk, N.; Ellis, K.; van Leeuwen, J.; van Heusden, S.; Kuijper, S.; Kolk, A. H. J.

CS R. Trop. Inst., Acad. Med. Cent., Amsterdam, Neth.

SO J. Clin. Microbiol. (1994), 32(3), 672-8

CODEN: JCMIDW; ISSN: 0095-1137

DT Journal

LA English

AB Diagnostic techniques based on PCR have two major problems: false-pos. reactions due to contamination with DNA fragments from previous PCRs (amplicons) and false-neg. reactions caused by inhibitors that interfere with the PCR. We have improved our previously reported PCR based on the amplification of a fragment of the Mycobacterium tuberculosis complex-specific insertion element IS6110 with respect to both problems. False-pos. reactions caused

by amplicon contamination were prevented by the use of uracil-N-glycosylase and dUTP instead of dTTP. We selected a new set of primers outside the region spanned by the formerly used primers to avoid false-pos. reactions caused by dTTP-contg. amplicons still present in the lab. With this new primer set, 16 copies of the IS6110 insertion element, the equiv. of two bacteria, could be amplified 1010 times in 40 cycles, resulting in a mean efficiency of 77% per cycle. To detect the presence of inhibitors of the **Taq polymerase**, which may cause false-neg. reactions, part of each sample was spiked with M. tuberculosis DNA. The DNA puritin. method using guanidinium thiocyanate and diatoms effectively removed most or all inhibitors of the PCR. However, this was not suitable for blood samples, for which we developed a proteinase K treatment followed by phenol-chloroform extn. This method permitted detection of 20 M. tuberculosis bacteria per ml of whole blood. Various lab. procedures were introduced to reduce failure or inhibition of PCR and avoid DNA cross contamination. We have tested 218 different clin. specimens obtained from patients suspected of having tuberculosis. The samples included sputum (n = 145), tissue biopsy samples (n = 25), cerebrospinal fluid (n = 15), blood (n = 14), pleural fluid (n = 9), feces (n = 7), fluid from fistulae (n = 2), and pus from a wound (n = 1). The results obtained by PCR were consistent with those obtained with culture, which is the "gold std.". We demonstrate that PCR is a useful technique for the rapid diagnosis of tuberculosis at various sites.

IT 1173-82-6, DUTP
 RL: ARG (Analytical reagent use); BUV (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (uses)
 (in PCR reactions for control of false-positives; a more reliable PCR for detection of Mycobacterium tuberculosis in clin. samples)

L36 ANSWER 2 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 2
 AN 120:155013 CA
 TI Production of hybridization probes by the PCR utilizing digoxigenin-modified nucleotides
 AU McCreery, Tom; Helentjaris, Tim
 CS Dep. Plant Sci., Univ. Arizona, Tucson, AZ, USA
 SO Methods Mol. Biol. (Totowa, N. J.) (1994), 28(Protocols for Nucleic Acid Analysis by Nonradioactive Probes), 67-71
 DT Journal
 LA English
 AB A method for the prepn. of digoxigenin-labeled DNA probes is described. Digoxigenin-11-dUTP is a good substrate for the **Taq polymerase** making PCR a preferred method for the prepn. of hybridization probes.

L36 ANSWER 3 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 3
 AN 123:1988 CA
 TI Polymerase chain reaction-based diagnostic assay to detect cattle chronically infected with Babesia bovis

AU Figueroa, J. V.; Chieves, L. P.; Johnson, G. S.; Goff, W. L.;
 Buening, G. M.
 CS Dep. Veterinary Microbiol., UMC, Columbia, MO, 65211, USA
 SO Rev. Latinoam. Microbiol. (1994), 36(1), 47-55
 CODEN: RLMIAA; ISSN: 0034-9771
 DT Journal
 LA English
 AB From a *B. bovis* gene sequence coding for a 60 kDa merozoite surface protein previously published, two sets of primers were designed for the Polymerase Chain Reaction (PCR) assay. Primer set BoF/BoR was used to prime **Taq Polymerase** DNA amplification of a 350 bp fragment of the target *B. bovis* DNA. Primer set BoFN/BoRN was used to prep. a PCR-synthesized, Digoxigenin-dUTP-labeled probe (291 bp) which would hybridize to a sequence within the PCR-amplified parasite target DNA. PCR amplification of target DNA obtained from in vitro-cultured *B. bovis* and nucleic acid hybridization of amplified product with the nonradioactive DNA probe showed that a 350 bp fragment could be detected when as little as 10 pg of genomic parasite DNA was utilized in the assay. A fragment of similar size was amplified from genomic DNA from four other *B. bovis* isolates but not from *B. bigemina*, *Anaplasma marginale*, or bovine leukocyte DNA. The PCR product was detected in blood samples contg. approx. 3 *B. bovis*-infected erythrocytes (20 .mu.L of packed cells with a parasitemia of 0.000001%). By using the PCR/DNA probe assay, 16 out of 20 animals exptl. inoculated with *B. bovis* were detected pos., whereas no PCR product was obsd. in bovine blood samples collected from 20 *B. bigemina*-infected, and 20 uninfected cattle tested. The PCR-DNA probe assay was shown to be sensitive in detecting some cattle with *B. bovis*-chronic infection. The specificity and high anal. sensitivity of the test provides a valuable tool to apply in conducting epidemiol. studies.

L36 ANSWER 4 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 4
 AN 118:185109 CA
 TI Novel amplification method for polynucleotide assays
 IN Dattagupta, Nanibhushan; Sullivan, Elizabeth C.
 PA Miles Inc., USA
 SO Eur. Pat. Appl., 7 pp.
 CODEN: EPXXDW
 PI EP 530526 A1 930310
 DS R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE
 AI EP 92-113394 920806
 PRAI US 91-744548 910813
 DT Patent
 LA English
 AB A nucleic acid sequence is detected in a sample by (1) treating the sample under hybridization conditions with an oligonucleotide that lacks a recognition site for enzyme digestion, (2) extending the hybridization product by adding polymerase and nucleoside triphosphates to create, on the oligonucleotide strand, a recognition site for enzyme digestion, (3) hybridizing the

oligonucleotide strand to a labeled probe which is immobilized or immobilizable and contains a recognition site for enzyme digestion that is completely or partially complementary to the recognition site for enzyme digestion on the oligonucleotide strand, (4) digesting the hybridization product with restriction endonuclease, and (5) detecting the sepd. label which is released in soln. A kit for detection of a nucleic acid sequence comprises a labeled probe, an oligonucleotide sequence for extension, and a restriction endonuclease. Thus, Chlamydia DNA was detected by use of a synthetic 22-mer oligonucleotide representing a portion of the gene for the major outer membrane protein of C. trachomatis, which was 5' end labeled with 32P using T4 polynucleotide kinase and 3' end labeled with biotin by thermocycling 30 times in the presence of biotin-11-dUTP, Taq polymerase, and a DNA sample. The amplified product was immobilized on streptavidin-coated magnetic particles, hybridized with a single-stranded complementary oligonucleotide contg. a restriction site for AluI, digested with AluI, and released radioactivity was detd. in the supernatant, after magnetic particle sepn., by denaturing gel electrophoresis. Extension of the labeled probe occurred in a specific manner, limited by the complementarity of the hybridizing sequence.

L36

ANSWER 5 OF 14 CA COPYRIGHT 1996 ACS

DUPLICATE 5

TI

Incorporation of dITP or 7-deaza dGTP during PCR

AU

Improves sequencing of the product
Dierick, Herman; Stul, Michel; De Kever, Wim; Marynen, Peter;

CS

Cent. Hum. Genet., Louvain, B-3000, Belg.

SO

Nucleic Acids Res. (1993), 21(18), 4427-8

DT

Journal

LA

English

AB

Premature enzyme pausing due to regions of complex secondary structure interferes with sequencing reactions. 7-

Deaza dGTP or the more economical dITP analogs were used to generate PCR fragments contg. one of these analogs instead of, or together with, dGTP. The isolated single strand template mols. showed less (than conventional PCR) or no secondary structures, allowing T7 polymerase to read through the entire sequence. Specific expt. conditions required to prevent dITP incorporation, which decreases PCR efficiency and increases misincorporation by Taq polymerase, are given. The method was applied to the sequencing of short fragments contg. exon 7 of the p53 gene and of the D-loop region of mitochondrial DNA.

L36

ANSWER 6 OF 14 CA COPYRIGHT 1996 ACS

DUPLICATE 6

AN

120:155055 CA

TI

Cycle sequencing protocol with fluorescein-12-dCTP

AU

Zimmermann, J.; Voss, H.; Wiemann, S.; Erle, H.; Rupp, T.; Hewitt,

CS

Europ. Mol. Biol. Lab., Heidelberg, D-6900, Germany

SO Methods Mol. Cell. Biol. (1993), 4(1), 27-28
CODEN: MMCBEV; ISSN: 0898-7750

DT Journal

LA English

AB A cycle sequencing protocol is reported which uses fluorescein-12-dCTP for direct sequencing of M13, plasmid, and cosmid DNA. This protocol results in better signal intensities and resolu. than do those employing fluorescein-12-dUTP. Fluorescein-15-dATP is not well accepted by **Taq polymerase** under conditions of this protocol. Automated base calling can be performed by com. available software.

L36 ANSWER 7 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 7

AN 119:155382 CA

TI Site-specific incorporation of [125I]iododeoxyuridine into DNA

AU Scherberg, Neal; Bloch, Isaac; Gardner, Paul

CS Univ. Chicago Hosp. Clin., Chicago, IL, 60637, USA

SO Appl. Radiat. Isot. (1992), 43(7), 923-7

CODEN: ARISEF; ISSN: 0883-2889

DT Journal

LA English

AB A procedure for the incorporation of [125I]IdU into specific sites in DNA is described. The approach depends upon attachment of radioiododeoxyuridine to a controlled pore glass support which is then used for automated synthesis of an oligomer. The resulting oligomer, contg. a terminal 3'[125I]iododeoxyuridine, is used as a primer during DNA synthesis catalyzed by the **Taq polymerase** employing thermal cycling. The product formed includes the radioiodonucleotide at a single internal site detd. by the length of the oligomer.

L36 ANSWER 8 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 8

AN 116:208756 CA

TI **7-Deazapurine** containing DNA: efficiency of c7GdTP, c7AdTP and c7IdTP incorporation during PCR-amplification and protection from endodeoxyribonuclease hydrolysis

AU Seela, Frank; Roeling, Angelika

CS Lab. Org. Bioorg. Chem., Univ. Osnabrueck, Osnabrueck, D-4500, Germany

SO Nucleic Acids Res. (1992), 20(1), 55-61

CODEN: NARHAD; ISSN: 0305-1048

DT Journal

LA English

AB The enzymic synthesis of **7-deazapurine** nucleoside-contg. DNA (501 bp) is performed by PCR-amplification (**Taq polymerase**) using a pUC18 plasmid DNA as template and the triphosphates of **7-deaza** -2'-deoxyguanosine (c7Gd), -adenosine (c7Ad) and -inosine (c7Id). c7GdTP can fully replace dGTP resulting in a completely modified DNA-fragment of defined size and sequence. The other two **7-deazapurine** triphosphates (c7AdTP) and (c7IdTP) require the presence of the parent purine 2'-deoxyribonucleotides. In purine/**7-deazapurine** nucleotide mixts.

Taq polymerase prefers purine over 7-deazapurine nucleotides but accepts c7GdTP much better than c7AdTP or c7IdTP. As incorporation of 7-deazapurine nucleotides represents a modification of the major groove of DNA it can be used to probe DNA/protein interaction. Regioselective phosphodiester hydrolysis of the modified DNA fragments was studied with 28 endonucleases. C7Gd is able to protect the DNA from the phosphodiester hydrolysis in more than 20 cases, only a few enzymes (Mae III, Rsa I, Hind III, Pvu II or Tag I) still hydrolyze the modified DNA. C7Ad protects DNA less efficiently, as this DNA could only be modified in part. The absence of N-7 as potential binding position or a geometric distortion of the recognition duplex caused by the 7-deazapurine base can account for protection of hydrolysis.

L36 ANSWER 9 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 9

AN 116:100248 CA
 TI Sequencing self-ligated PCR products using 3' over-hangs generated by specific cleavage of dUTP by uracil-DNA glycosylase
 AU Day, P. J. R.; Walker, M. R.
 CS Dep. oncol., Child. Hosp., Birmingham, B16 8ET, UK
 SO Nucleic Acids Res. (1991), 19(24), 6959
 DT JOURNAL
 LA English
 AB

PCR products were self-ligated using homopolymer tails generated by the inclusion of 5' polyadenosine and polythymidine sequences followed by deoxyuracil (dUTP) to the forward and reverse primer sequences resp. dUTP was specifically excised from the PCR primers by using uracil-DNA glycosylase to leave complementary sequences to the homopolymer sequences single-stranded. The homopolymer sequences were chosen to be complementary, and were used to facilitate efficient ligation and subsequent sequencing of 120 bp and 710 bp PCR products. Control reactions omitting ligase did not produce a usable sequencing template. Therefore, because concatamerization was not observed, the conformation of PCR product formed in the presence of DNA ligase must reduce the rate of PCR product reannealing, thus assisting annealing of the sequencing primer and enabling direct sequencing from either PCR strand. The introduction of an A-A mismatch does not impose a problem for ligation, presumably due to the clamping produced by the polynucleotide sequence. Also, the poor fidelity with which polymerases read dUTP may actually increase the likelihood of correctly matched sequences, although this effect has not been observed. By introducing one or more over-hanging bases into PCR products (as depicted by primer sequence 5' to the uracil moiety), all amplified products will have the appropriate over-hang, rather than relying on the chance probability of non-template directed 3' incorporation of adenosine by Taq polymerase.

polymers.

RL: USES (Uses)

(of polymerase chain reaction products, uracil-DNA glycosylase)

specific cleavage of, DNA sequence detn. in relation to)

- L36 ANSWER 10 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 10
AN 115:200321 CA
TI Improved telomere detection using a telomere repeat probe (TTAGGG)_n generated by PCR
AU IJdo, J. W.; Wells, R. A.; Baldini, A.; Reeders, S. T.
CS Howard Hughes Med. Inst., New Haven, CT, 06510, USA
SO Nucleic Acids Res. (1991), 19(17), 4780
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB The rapid generation of human telomere repeat sequence (TTAGGG)_n, with fragment sizes up to 25 kb using the polymerase chain reaction (PCR) is reported. This probe can be labeled with biotin-11-dUTP or a mixt. of modified eoxynucleotides in the same PCR reaction. Fluorescence in situ hybridization shows signal at all telomers with a signal intensity significantly stronger than that seen using an oligonucleotide probe of the same sequence. The (TTAGGG)_n probe is also a useful tool as an anchor point in fluorescence in situ expts. in which several probes are used simultaneously. It allows merging of the different images obtained with several probes labeled with different fluorochromes at the same time. PCR is carried out in the absence of template using primers (TTAGGG)₅ and (CCCTAA)₅. Staggered annealing of the primers provides a single strand template for extension by **Taq polymerase**. The primers serve as template in the early PCR cycles whereas the newly formed templates serve as primer and template in subsequent stages of the PCR, resulting in a heterogeneous population of mols. consisting of repeat arrays of various lengths. Clearly, reducing the initial primer concn. increases the av. length of the products.
- L36 ANSWER 11 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 11
AN 115:130926 CA
TI Producing single-stranded DNA probes with the Taq DNA polymerase: a high yield protocol
AU Finckh, Ulrich; Lingenfelter, Patricia A.; Myerson, David
CS Fred Hutchinson Cancer Res. Cent., Seattle, WA, 98104, USA
SO BioTechniques (1991), 10(1), 35-6, 38
CODEN: BTNQDO; ISSN: 0736-6205
DT Journal
LA English
AB An efficient procedure is reported to synthesize either single- or double-stranded probes labeled with biotin-11dUTP, biotin-21-dUTP or digoxigenin-11dUTP. To produce the single-stranded probe, only a single primer is utilized in a **Taq polymerase** amplification of 55 cycles. A cytomegalovirus probe is presented. This procedure allows easy prodn. of nonradioactivity labeled pure single-stranded probes of any desired length and specificity.

L36 ANSWER 12 OF 14 CA COPYRIGHT 1996 ACS DUPLICATE 12

AN 114:18981 CA
TI Structure-independent DNA amplification by the polymerase chain
reaction using 7-deaza guanine

IN Innis, Michael A.
PA Cetus Corp., USA
SO PCT Int. Appl., 16 pp.
CODEN: PIXXD2
PI WO 9003443 A1 900405
DS W: AU, JP
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE
AI WO 89-US4100 890919
PRAI US 88-248556 880923
DT Patent
LA English
AB DNA sequences that form stable secondary structures, e.g. hairpin loops, and so are not efficiently amplified in std. polymerase chain reaction incubations are efficiently amplified when dGTP is partly replaced by 7-deazadeoxyguanine triphosphate.
Optimization expts. using the murine ornithine decarboxylase gene showed that a partial substitution of dGTP gave the most efficient results. The amplification of a cDNA for human beta-actin was also demonstrated.

L36 ANSWER 13 OF 14 CA COPYRIGHT 1996 ACS
AN 114:18884 CA
TI Preparation of digoxigenin-labeled probes by the polymerase chain reaction

AU Lanzillo, Joseph J.
CS Pulm. Div., New England Med. Cent., Boston, MA, 02111, USA
SO Biotechniques (1990), 8(6), 620, 622
CODEN: BTNQDO; ISSN: 0736-6205
DT Journal
LA English
AB Because of the health hazard, cost and instability assocd. with radio-labeled probes, it is anticipated that they will be replaced ultimately with nonradioactive probes. It is reported here that digoxigenin can be introduced into probes efficiently with digoxigenin-11-dUTP (Dg-11-dUTP, Boehringer Mannheim, Indianapolis, IN) by the polymerase chain reaction (PCR). Digoxigenin was readily incorporated into the 480 bp-amplified fragment by Taq polymerase. Dot blot hybridization with the 50% Dg-labeled probe readily detected 1 pg target DNA by direct chemiluminescence after a 1-h exposure of the blot to x-ray film.

L36 ANSWER 14 OF 14 CA COPYRIGHT 1996 ACS
AN 112:193185 CA
TI Sequencing with Taq DNA polymerase

AU Brow, Mary Ann D.
CS McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706, USA
SO PCR Protoc.: Guide Methods Appl. (1990), 189-96. Editor(s): Innis, Michael A. Publisher: Academic, San Diego, Calif.
CODEN: 56TMAY

DT Conference
LA English
AB Taq DNA polymerase has proven to be highly advantageous for the dideoxynucleotide chain-termination method of DNA sequencing of both conventional and single-stranded PCR templates. The basic sequencing protocol described here involves (1) annealing an oligonucleotide primer to a single-stranded template; (2) labeling the primer in a short, low-temp. polymn. reaction in the presence of .alpha.-labeled dNTP and 3 unlabeled dNTPs, all at low concn.; and (3) extending the labeled primer in 4 sep. base-specific, high-temp. reactions, each in the presence of higher concns. of all dNTPs and 1 chain-terminating ddNTP. If 5'-end-labeled primers are used, step (2) is eliminated. The helix-destabilizing base analog 7-deaza-2'-deoxyguanosine-5'-triphosphate (c7dGTP) can be incorporated to prevent gel compressions. The products of these reactions are then sepd. by high-resoln. polyacrylamide-urea gel electrophoresis and visualized by autoradiog. or by nonisotopic detection methods.

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L39 6 FILE EMBASE
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L40 3 FILE LIFESCI

L41 1 FILE BIOTECHDS
 'CN' IS NOT A VALID FIELD CODE 2 FILE WPIDS
 L42 'CN' IS NOT A VALID FIELD CODE
 L43 'CN' IS NOT A VALID FIELD CODE 0 FILE CONFSCI
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=> d 1-19 bib abs; fill ca, caplus

L47 ANSWER 1 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 1
 AN 94:161547 BIOSIS
 DN 97174547

TI A more reliable PCR for detection of Mycobacterium tuberculosis in

clinical samples.

AU Kox L F F; Rhienthong D; Miranda A M; Udomasantsuk N; Ellis K; Van

Leeuwen J; Van Heusden S; Kuijper S; Kolk A H J

CS Royal Tropical Inst., N.H. Sullengrebel Lab. Tropical Hygiene,

Weibergdref 39, 1105 AZ Amsterdam, NET

SO Journal of Clinical Microbiology 32 (3). 1994. 672-678. ISSN:

0095-1137

LA English

AB

Diagnostic techniques based on PCR have two major problems:

false-positive reactions due to contamination with DNA fragments from previous PCRs (amplicons) and false-negative reactions caused by inhibitors that interfere with the PCR. We have improved our previously reported PCR based on the amplification of a fragment of the Mycobacterium tuberculosis complex-specific insertion element IS6110 with respect to both problems. False-positive reactions caused by amplicon contamination were prevented by the use of uracil-N-glycosylase and dUTP instead of dTTP. We selected a new set of primers outside the region spanned by the formerly used primers to avoid false-positive reactions caused by dTTP-containing amplicons still present in the laboratory. With this new primer set, 16 copies of the IS6110 insertion element, the equivalent of two bacteria, could be amplified 10-10 times in 40 cycles, resulting in a mean efficiency of 77% per cycle. To detect the presence of inhibitors of the Taq polymerase, which may cause false-negative reactions, part of each sample was spiked with M. tuberculosis DNA. The DNA purification method using guanidinium thiocyanate and diatoms effectively removed most or all inhibitors of the PCR. However, this was not suitable for blood samples, for which we developed a proteinase K treatment followed by phenol-chloroform extraction. This method permitted detection of 20 M. tuberculosis

bacteria per ml of whole blood. Various laboratory procedures were introduced to reduce failure or inhibition of PCR and avoid DNA cross contamination. We have tested 218 different clinical specimens obtained from patients suspected of having tuberculosis. The samples included sputum (n = 145), tissue biopsy samples (n = 25), cerebrospinal fluid (n = 15), blood (n = 14), pleural fluid (n = 9), feces (n = 7), fluid from fistulae (n = 2), and pus from a wound (n = 1). The results obtained by PCR were consistent with those obtained with culture, which is the "gold standard." We demonstrate that PCR is a useful technique for the rapid diagnosis of tuberculosis at various sites.

L47 ANSWER 2 OF 19 MEDLINE

AN 94325338 MEDLINE

TI Comparison of the sequence specificity of cis-diamminedichloroplatinum (II) damage in guanine- and 7-deazaguanine-containing DNA.

AU Cairns M J; Murray V

CS School of Biochemistry and Molecular Genetics, University of New South Wales, Kensington, Australia..

SO BIOCHIMICA ET BIOPHYSICA ACTA, (1994 Aug 2) 1218 (3) 315-21.
Journal code: AOW. ISSN: 0006-3002.

CY Netherlands

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 9411

AB The N7 of guanine is thought to be the primary target for adduct and crosslink formation between cisplatin and DNA. However, reactive sites in DNA other than the N7 of guanine may also participate in the formation of adducts with cisplatin. The possibility that these interactions arise and form DNA polymerase blocking lesions was investigated by primer extension reactions with Taq DNA polymerase. To differentiate between damage produced at relatively weak sites from those formed at the N7 of guanine, a modified DNA template was synthesised with the N7 of guanine replaced with a carbon atom. This was achieved in a PCR designed to incorporate 7-

deazaguanine instead of normal guanine. The sequence specificity of cisplatin damage in the modified and unmodified DNA substrates was compared (after linear amplification) by DNA sequencing gel analysis. For concentrations of cisplatin (1 to 5 microM) that induce blocking lesions in normal DNA, no significant damage was observed in the modified DNA. This confirmed that the N7 of guanine is the major site of adduct formation in normal DNA. At higher concentrations of cisplatin (50 microM and 100 microM), lesions were found at AA dinucleotides and other novel sites in the modified DNA. These results indicate that the N7 of guanine is not required in the formation of some cisplatin adducts.

L47 ANSWER 3 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2

AN 95:58783 BIOSIS

DN 98073083

TI In situ transcription with Tth DNA polymerase and fluorescent

AB nucleotides.
 CS Naval Med. Res. Inst., Code 63, Bethesda, MD 20889-5607, USA
 SO Journal of Immunological Methods 176 (2): 1994. 235-243. ISSN: 0022-1759
 LA English

We and others have described methods to label specific nucleic acid sequences in fixed cells by reverse in situ transcription (IST). They are simple alternatives to the tedious steps of in situ hybridization with labeled probes. We have favored use of thermostable DNA polymerases after heat denaturation of template secondary structure, accompanied by synthesis of cDNA from an annealed primer, but the approach has been limited by the low reverse transcriptase (RT) activity of **Taq polymerase** and delayed detection methods. We have improved the technique by the use of recombinant **Thermus thermophilus** (Tth) DNA polymerase and fluorescein-12-dUTP (FIST). Jurkat T lymphocytes were stimulated with ionomycin + phorbol myristate acetate to produce interleukin-2 (IL-2) mRNA in vitro overnight. They were cytospun onto slides and fixed in 70% ethanol + 30% DEPC-treated water, acetone, and air-dried. The slides were placed on a temperature-controlled heating block, and the cell spot was covered with a plastic coverslip. The temperature was raised to 95 degree C, and 5-10 µl of modified Perkin-Elmer/Cetus RT reaction mix was injected under the edge of the coverslip. Each 10 µl of mix in DEPC-water contained 10 mM Tris-HCl, pH 8.3, 90 mM KCl, 1 mM MnCl₂, 1 mM dithiothreitol, 10 U placental ribonuclease inhibitor, 0.125 mM dA, C, GTPS, 0.1 mM fluorescein-12-dUTP, 2 U Tth DNA polymerase, and 4 µM 22-mer oligonucleotide primer, which spanned the second intron of IL-2. After 3 min at 95 degree C, 1 min at 50 degree C and 10 min at 72 degree C, the slides were washed in 0.5 times phosphate-buffered saline, pH 7.0, at 42 degree C, in 70% ethanol, 100% ethanol, and air-dried. The cells were mounted in antifade solution (2% n-propyl gallate in 70% glycerol), and could be viewed immediately by fluorescence microscopy. Image analysis showed that stimulated Jurkat cells were brighter than uninduced controls or those treated with RNase or without polymerase or primer. FIST appears to be useful for the detection of specific mRNAs in single cells.

L47 ANSWER 4 OF 19 MEDLINE
 AN 94367331 MEDLINE
 TI Use of a nonradioactive genetic probe identified, synthesized, and labeled in the polymerase chain reaction.
 AU Preus H R; Russell D T
 CS Department of Periodontology, Dental Faculty, University of Oslo, Norway.
 SO SCANDINAVIAN JOURNAL OF DENTAL RESEARCH, (1994 Jun) 102 (3) 161-7.
 CY Denmark
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals; Dental Journals
 EM 9412

AB This study introduces a strategy to identify and produce sequences useful as genetic markers, or native genetic probes for DNA-DNA hybridization in bacterial strains where the genetics is not well described. *Actinobacillus actinomy-cetemcomitans* (A.a.) was used as an example. Fifty ng genomic DNA from A.a. ATCC 33384 and *Haemophilus aphrophilus* ATCC 33389 was amplified in a thermocycler using a single 10-mer primer. The PCR products were separated by electrophoresis on a 1% submarine agarose gel containing ethidium bromide and visualized by UV illumination, and the strain-specific amplicons were compared. DNA from two bands, 0.9 and 4 kb, unique for the A.a. strain, was cut out, amplified under high stringency with the same primer and labeled by replacing 33.3 microM dTTP with digoxigenin-labeled dUTP in the reaction mixture. The labeled probe was then repeatedly used for hybridization to DNA from various A.a., *H. aphrophilus*, and other bacterial strains of the Pasteurellaceae family. The results showed that the 0.9-kb probe detected all A.a. tested, and distinguished it from other closely related bacterial species. We conclude that the described strategy is useful for identifying and selecting genetic sequences useful as genetic markers in A.a.

L47 ANSWER 5 OF 19 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.

AN 94216013 EMBASE

TI [Intracellular PCR: A new approach for diagnoses].

PCR INTRACELLULAIRE: NOUVELLE APPROCHE DE DIAGNOSTIC TISSULAIRE ET CYTOGENETIQUE.

AU Teyssier M.; Carosella E.; Gluckman E.; Kirszenbaum M.

CS CEA, Laboratoire d'Immunoradiobiologie, Hopital Saint-Louis, 1, Avenue Claude-Vellefaux, 75475 Paris Cedex 10, France

SO IMMUNO-ANAL. BIOL. SPEC., (1994) 9/3 (159-164).

ISSN: 0923-2532 CODEN: IBSPEW

CY France

DT Journal

FS 022 Human Genetics

026 Immunology, Serology and Transplantation

LA French

SL French; English

AB The aim of this study was to improve the technique of in situ polymerase chain reaction (PCR) on glass slides in order to detect CD34 and c-kit genes on individual mononuclear cells (MNC). This method appears to increase the sensitivity of ISH without loss of morphology. MNC were deposited on slides, fixed and permeabilized. After in situ PCR, hybridization was realized with a digoxigenin-dUTP(DIG)-labelled probe. Detection was mediated by an anti-DIG antibody conjugate to alkaline phosphatase and the color substrate of this enzyme. It is worthy to mention that some PCR products diffused out of the cells in which amplification occurs and could be detected by electrophoresis of the supernatant. This direct qualitative control approach led to rapid visualization of amplified products before subsequent ISH. The PCR positive cells were identified by a black-blue coloration, whereas with the same hybridization, cells that undergone PCR without Taq polymerase remained uncolored.

L47 ANSWER 6 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 3

AN

94:440030 BIOSIS

DN 97453030

TI Polymerase chain reaction-based diagnostic assay to detect cattle

chronically infected with Babesia bovis.

AU Figueroa J V; Chaves L P; Johnson G S; Goff W L; Buning G M

CS CENID-PAVET, INIFAP-SARH. Apdo. Postal No. 206 CIVAC, Morelos, 62500,

MEX

SO Revista Latinoamericana de Microbiologia 36 (1). 1994. 47-55. ISSN:

0034-9771

LA English

AB From a B. bovis gene sequence coding for a 60 kDa merozoite surface

protein previously published, two sets of primers were designed for the Polymerase Chain Reaction (PCR) assay. Primer set Bof/Bor was used to prime **Taq Polymerase** DNA amplification of

a 350 bp fragment of the target B. bovis DNA. Primer set BofN/BORN was used to prepare a PCR-synthesized, Digoxigenin-dUTP

-labeled probe (291 bp) which would hybridize to a sequence within the PCR-amplified parasite target DNA. PCR amplification of target

DNA obtained from in vitro-cultured B. bovis and nucleic acid hybridization of amplified product with the nonradioactive DNA probe

showed that a 350 bp fragment could be detected when as little as 10 pg of genomic parasite DNA was utilized in the assay. A fragment of

similar size was amplified from genomic DNA from four other B. bovis isolates but not from B. bigemina, Anaplasma marginale, or bovine

leukocyte DNA. The PCR product was detected in blood samples containing approximately 3 B. bovis-infected erythrocytes (20 mu-l of

packed cells with a parasitemia of 0.00001%). By using the PCR/DNA probe assay, 16 out of 20 animals experimentally inoculated with B.

bovis were detected positive, whereas no PCR product was observed in bovine blood sample collected from 20 B. bigemina-infected, and 20

uninfected cattle tested. The PCR-DNA probe assay was shown to be sensitive in detecting some cattle with B. bovis-chronic infection.

The specificity and high analytical sensitivity of the test provides a valuable tool to apply in conducting epidemiological studies.

L47

ANSWER 7 OF 19 MEDLINE

AN

93324337 MEDLINE

TI

Elimination of band compression in sequencing gels by the use of

N4-methyl-2'-deoxycytidine 5'-triphosphate.

AU

Li S; Haces A; Stupar L; Gebeyehu G; Pless R C

CS

Life Technologies, Inc., Gaithersburg, MD 20884-9980..

SO

NUCLEIC ACIDS RESEARCH, (1993 Jun 11) 21 (11) 2709-14.

CY

Journal code: 08L. ISSN: 0305-1048.

DT

Journal: Article; (JOURNAL ARTICLE)

FS

Priority Journals; Cancer Journals

EM

9310

Tag DNA polymerase, Sequenase, and the large fragment of E.coli

polymerase I effectively utilize N4-methyl-2'-deoxycytidine

5'-triphosphate (N4-methyl-dCTP) in the place of dCTP in

dideoxynucleotide terminator sequencing reactions on single-stranded templates. When the resulting fragment mixtures are resolved on sequencing gels, they are found to be free of band compressions even in cases where such compressions remain unresolved by the substitution of 7-deaza-dGTP for dGTP.

Sequencing reactions using N4-methyl-dCTP instead of dCTP are somewhat more prone to false stops than are sequencing reactions using 7-deaza-dGTP instead of dGTP; this difference is more pronounced when sequencing with Sequenase at 37 degrees C than when sequencing with Taq DNA polymerase at 72 degrees C. For the three polymerases investigated, replacement of dCTP by N4-methyl-dCTP does not fundamentally change the characteristic variations in band intensities seen in the C-lane. N4-methyl-dCTP can also be used for sequencing double-stranded DNA and for DNA amplification by the polymerase chain reaction.

L47 ANSWER 8 OF 19 MEDLINE

AN 93356464 MEDLINE

TI Pitfalls of in situ polymerase chain reaction (PCR) using direct incorporation of labelled nucleotides.

AU Sallstrom J F; Zehbe I; Alemi M; Wilander E

CS Department of Pathology, University Hospital, Uppsala, Sweden..

SO ANTICANCER RESEARCH, (1993 Jul-Aug) 13 (4) 1153.

Journal code: 59L. ISSN: 0250-7005.

CY Greece

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

EM 9311

AB False positivity is reported of in situ PCR reactions in a direct incorporation assay with digoxigenin-labelled dUTP. It is recommended that in situ hybridization with specific labelled probe replaces the direct incorporation method for the detection of in situ PCR amplicon.

L47 ANSWER 9 OF 19 MEDLINE

AN 93207760 MEDLINE

TI Overcoming GC compression in nucleotide sequencing.

AU Beck K F; Stathopoulos I; Berninger M G; Schweizer M

CS Institut fur Mikrobiologie und Biochemie, Universitat Erlangen-Nurnberg, FRG..

SO BIOTECHNIQUES, (1993 Mar) 14 (3) 375.

Journal code: AN3. ISSN: 0736-6205.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 9307

L47 ANSWER 10 OF 19 LIFESCI COPYRIGHT 1996 CSA

AN 93:132239 LIFESCI

TI Cycle sequencing protocol with fluorescein-12-dCTP.

AU Zimmermann, J.; Voss, H.; Wiemann, S.; Erfle, H.; Rupp, T.; Hewitt,

CS N.A.! Schwager, C.! Stegemann, J.! Ansgar, W.
Eur. Mol. Biol. Lab., D-6900 Heidelberg, FRG
METHODS MOL. CELL. BIOL., (1993) Vol. 4, no. 1, pp. 27-28.
ISSN: 0898-7750.

DT Journal
FS N! G! G3
LA English
AB

Recently we have introduced procedures for automated DNA sequencing with internal labeling and T7 DNA polymerase, using fluorescein-12-dUTP and fluorescein-15-dATP without the need of labeled primers or fluorescent dye terminators. Cycle sequencing protocols were developed for automated DNA sequencing, allowing the sequencing of small amounts of DNA. The principle of internal labeling for cycle sequencing with Taq DNA polymerase was shown with fluorescein-12-dUTP. Here we report a cycle sequencing protocol using the fluorescein-12-dCTP, which we applied to direct sequencing of M13, plasmid, and cosmid DNA. This protocol results in better signal intensities and resolution than those employing fluorescein-12-dUTP. Fluorescein-15-dATP is not well accepted by Taq polymerase under conditions of this protocol. Automated base calling can be performed by commercially available software (A.L.F., Pharmacia).

L47 ANSWER 11 OF 19 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN WPIDS

CR 92-064970 [08]; 89-233845 [32]; 91-222898 [30]; 91-222902 [30]; 92-096899 [12]; 92-150885 [18]; 92-150887 [18]; 94-150436 [18]; 95-154582 [20]; C92-029827

TI Reducing non-specific nucleic acid amplification - by inserting

modified nucleic acid bases into reaction mixt and treating prods. to prevent their action as templates.

DC B04 D16

IN GELFAND, D H; KWOK, S Y; SNINSKY, J J
PA (CETU) CETUS CORP; (HOFF) HOFFMANN LA ROCHE & CO AG F; (HOFF)

CYC 18

PI WO 9201814 A 920206 (9208)*

RW: AT BE CH DE DK ES FR GB GR IT LU NL SE
W: AU CA JP US

AU 9185327 A 920218 (9222)
EP 540693 A1 930512 (9319) EN 56 pp

R: AT BE CH DE DK ES FR GB GR IT LU NL SE
JP 06501612 W 940224 (9413) 28 pp

US 5418149 A 950523 (9526) 23 pp
AU 665338 B 960104 (9608)

ADT

AU 9185327 A AU 91-85327 910723, WO 91-US5210 910723; EP 540693 A1
EP 91-916353 910723, WO 91-US5210 910723; JP 06501612 W JP 91-515604
910723, WO 91-US5210 910723; WO 9201814 A3 WO 91-US5210 910723; US
5418149 A CIP of US 90-557517 900724, CIP of US 90-609157 901102, WO
91-US5210 910723, US 93-960362 930105; AU 665338 B AU 91-85327

FDT AU 9185327 A Based on WO 9201814; EP 540693 A1 Based on WO 9201814;
JP 06501612 W Based on WO 9201814; US 5418149 A Based on WO 9201814;
AU 665338 B Previous Publ. AU 9185327, Based on WO 9201814

PRAI US 90-609157 901102; US 90-557517 900724; US 93-960362 930105

AN 92-064970 [08] WPIDS

CR 88-058187 [09]; 89-233845 [32]; 91-222898 [30]; 91-222902 [30];
92-096899 [12]; 92-150885 [18]; 92-150887 [18]; 94-150436 [18];
95-154582 [20]

AB WO 9201814 A UPAB: 931006

Reducing non-specific amplification in a primer-based amplification reaction comprises; (a) incorporating a modified nucleoside triphosphate (nTP) and a glycosylase specific for the nTP, in the reaction; (b) incubating the reaction of step (a) at a temp. below that of denaturation of the glycosylase and below that of specific hybridisation of the primers to render the modified nucleotides in the amplification prods. basic; (c) inactivating the glycosylase; and (d) incubating the reaction of step (c) at the temp. for specific hybridisation of the primers.

Also claimed are; (1) sterilising a nucleic acid (NA) amplification reaction system, comprising degrading the contaminating amplified prod. by hydrolysing covalent bonds of the unconventional nucleotides; (2) improved amplification methods, generating amplified NA that can be rendered unamplifiable, preventing deleterious effects from the amplified NA contaminating subsequent amplifications; (3) purifying a recombinant protein (A) from a host cell.

ADVANTAGE - Improved amplification methods can reduce non-specific amplification of NAs as well as minimising the effects of contamination with previously generated prods.. Methods allow an enhanced specificity of NA amplification assays. Na-free proteins can be produced which are useful as reagents for amplification systems.

0/0

ABEQ EP 540693 A UPAB: 931113

Reducing non-specific amplification in a primer based amplification reaction comprises: (a) incorporating a modified nucleoside triphosphate (nTp) and a glycosylase specific for the nTP, in the reaction; (b) incubating the reaction of step (a) at a temp. below that of denaturation of the glyosylase and below that of specific hybridisation of the primers to render the modified nucleotides in the amplification prods, basic; (c) inactivating the glycosylase; and (d) incubating the reaction of step (c) at temp. for specific hybridisation of the primers.

Also claimed are: (1) sterilising a nucleic acid (NA) amplification reaction system, comprising degrading the contaminating amplified prod. by hydrolysing covalent bonds of the unconventional nucleotides; (2) improved amplification methods, generating amplified NA that can be rendered unamplifiable, preventing deleterious effects from the amplified NA contaminating subsequent amplifications; and (3) purifying a recombinant protein (A) from a host cell.

ADVANTAGE - Improved amplification methods can reduce non-specific amplification of NAs as well as minimising the effects

of contamination with previously generated prods. Methods allow an enhanced specificity of NA amplification assays. Na-free proteins can be produced which are useful as reagents for amplification.

ABEQ US 5418149 A UPAB: 950705

Redn. of non-specific amplification in primer-based amplification comprises incubation of the amplification mixt. in the presence of a modified nucleoside triphosphate (dUTP) so that the synthetic nucleic acid contains a modified nucleotide and also a glycosylase (UNG) that degrades nucleic acid contg. the modified nucleotide at 45-60 deg.C until all nucleic acid contg. the modified nucleotide has been degraded, then further amplification at temps. above 55 deg.C. Pref. reaction are chain reactions involving a thermally stable DNA polymerase.

USE/ADVANTAGE - The process is applicable to nucleic acid synthesis by means of chain reactions with **Taq**

polymerase. The process avoids the accumulation of non-specific templates, giving synthetic nucleic acids of improved purity.

Dwg.0/0

L47

ANSWER 12 OF 19 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
92-13390 BIOTECHDS

AN

Improved efficiency in amplification of ancient DNA and its sequence analysis!

using the polymerase chain reaction

AU

Hummel S; Nordiek G; Herrmann B
Institut fuer Anthropologie der Universitaet, W-3400 Goettingen,

LO

Naturwissenschaften; (1992) 79, 8, 359-60

SO

CODEN: NATWAY

DT

Journal

LA

English

AN

92-13390 BIOTECHDS

AB

A modified 'booster' polymerase chain reaction (PCR) protocol was suggested for amplifying DNA from ancient sources. A first series of 10 cycles was performed with a regular primer concentration, regular dNTP content and regular, buffered reaction mix, with 1% of the DNA deriving from about 0.25 g of 4-day EDTA-extracted bone powder and 1.0 U of Taq DNA-polymerase (EC-2.7.7.7). The stringency of the reaction was low in these first 10 cycles. A second series of 20-25 cycles was performed with high stringency parameters, lowered primer concentrations and 1.5-2.0 U Taq polymerase, with 10% of the initial 50 uI reaction used as template. DNA extracted from ancient bones of females and males was processed. Primers specific for the Y-chromosome, yielded amplification products from all male but no female bone extracts. For cloning, these amplified products were treated with phage T7 DNA-polymerase, blunt-end ligated into vector phasmid pmc5-delta bla ac and transformed into Escherichia coli SURB. Automated sequencing was performed using T7 polymerase with internal labeling of single-stranded DNA using fluorescein-12-dUTP. (16)

ref)

L47 ANSWER 13 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 4
 AN 92:184752 BIOSIS
 DN BA93:95702
 TI **7 DEAZAPURINE** CONTAINING DNA EFFICIENCY OF
 C-7G-DTP C-7A-DTP AND C-7I-DTP INCORPORATION DURING PCR-AMPLIFICATION
 AND PROTECTION FROM ENDODEOXYRIBONUCLEASE HYDROLYSIS.
 AU SEELA F; ROELING A
 CS LAB. ORGANISCHE BIOORGANISCHE CHEMIE, FACHBEREICH BIOLOGIE/CHEMIE,
 UNIVERSITAET OSNABRUCK, BARBARASTRASSE 7, D-4500 OSNABRUCK, GER.
 SO NUCLEIC ACIDS RES 20 (1). 1992. 55-61. CODEN: NARHAD ISSN: 0305-1048
 LA English
 AB The enzymatic synthesis of **7-deazapurine**
 nucleoside containing DNA (501 bp) is performed by PCR-amplification
 (**Taq polymerase**) using a pUC18 plasmid DNA as
 template and the triphosphates of **7-deaza**
 -2'-deoxyguanosine (c7Gd), -adenosine (c7Ad) and -inosine (c7Id).
 c7GdTP can fully replace dGTP resulting in a completely modified
 DNA-fragment of defined size and sequence. The other two **7-**
deazapurine triphosphates (c7AdTP) and (c7dTP) require the
 presence of the parent purine 2'-deoxyribonucleotides. In purine/
7-deazapurine nucleotide mixtures **Taq**
polymerase prefers purine over **7-**
deazapurine nucleotides but accepts c7GdTP much better than
 c7AdTP or c7dTP. As incorporation of **7-deazapurine**
 nucleotides represents a modification of the major groove of DNA it
 can be used to probe DNA/protein interaction. Regioselective
 phosphodiester hydrolysis of the modified DNA-fragments was studied
 with 28 endodeoxyribonucleases. c7Gd is able to protect the DNA from
 the phosphodiester hydrolysis in more than 20 cases, only a few
 enzymes (Mae III, Rsa I, Hind III, Pru II or Taq I) do still
 hydrolyze the modified DNA. c7Ad protects DNA less efficiently, as
 this DNA could only be modified in part. The absence of N-7 as
 potential binding position or a geometric distortion of the
 recognition duplex caused by the **7-deazapurine**
 base can account for protection of hydrolysis.

L47 ANSWER 14 OF 19 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
 AN 91-095712 [14] WPIDS
 DNC C91-040924
 TI Nucleic acid hybridisation assays - using a capture probe
 immobilised on a solid support to bind a labelled target nucleic
 acid sequence.
 DC B04 D16
 IN LONGIARU, M; SILVER, S B; SULZINSKI, M A
 PA (HOFF) HOFFMANN LA ROCHE & CO AG F; (HOFF) HOFFMANN-LA ROCHE AG;
 (HOFF) HOFFMANN LA ROCHE INC
 CYC 21
 PI EP 420260 A 910403 (9114)*
 R: AT BE CH DE ES FR GB IT LI NL SE
 AU 9063290 A 910411 (9122)
 NO 9004240 A 910402 (9122)
 CA 2026280 A 910330 (9124)
 ZA 9007706 A 910626 (9132)

BR 9004881 A 910910 (9141)
 JP 03206898 A 910910 (9142)
 US 5232829 A 930803 (9332)
 NZ 235463 A 940325 (9426)
 NZ 247522 A 940325 (9426)
 IL 95800 A 941007 (9445)
 AU 9472804 A 941124 (9503)
 EP 420260 A EP 90-118620 900927; ZA 9007706 A ZA 90-7700 900926; JP
 03206898 A JP 90-262934 900929; US 5232829 A US 89-414542 890929; NZ
 235463 A NZ 90-235463 900926; NZ 247522 A NZ 90-247522 900926; IL
 95800 A IL 90-95800 900926; AU 9472804 A AU 94-72804 940902, Div ex
 AU 90-63290
 NZ 247522 A Div ex NZ 235463
 PRAI US 89-414542 890929
 AN 91-095712 [14] WPIDS
 AB EP 420260 A UPAB: 950110

(A) a method of detecting a labelled target nucleic acid sequence amplified from a biological sample comprising hybridising the target nucleic acid sequence with at least one oligonucleotide capture probe having a nucleic acid sequence complementary to the target sequence, the capture probe being bound to a polystyrene solid support, and determining the presence of the label associated with the target sequence; the hybridisation may be conducted in the presence of guanidine thiocyanate; the label may be biotin and the target sequence may be labelled by biotin-11-dUTP incorporation by Taq polymerase during

polymerase chain reaction (PCR) amplification; the biotin label may be detected by addn. of avidin or streptavidin complexed with horseradish peroxidase (HRP), alkaline phosphatase, beta-galactosidase, luciferase, fluorescein or Texas red; the HRP may be detected with a chromogenic agent, e.g. 3,3',5,5'-tetramethyl benzidine or o-phenylene diamine and a substrate, e.g. H2O2. (B) a method of quantitatively determining the extent of hybridisation of a target nucleic acid sequence in a capture probe assay which comprises (a) utilising microtitre plates as the support for passively bound capture probes, (b) contacting the plates with a biological sample contg. a target nucleic acid sequence which has been labelled to generate a signal capable of detection and (c) counting or otherwise measuring in quantitative fashion the signals so generated.

USE/ADVANTAGE - The method is used for the labelling and detection of target sequences, e.g. Chlamydia trachomatis nucleic acid. The plate assay is highly accurate and sensitive and can be accomplished in 2 hrs.. The capture of the labelled target sequences provides an objective, quantitative evaluation of hybridisation, the calculation of a statistical cut-off point for positivity of samples and the quantitative calculation of an assay "signal-to-noise" ratio. @ (21pp Dwg.No.0/3)

ABEQ US 5232829 A UPAB: 931118
 Amplified DNA is produced by a polymerase chain reaction, labelled (e.g. with biotin), then hybridised with a base pair to an amplicon-specific oligonucleotide that has been immobilised on a

solid polystyrene support, pref. on well walls in a microtitration plate.

USE - The process is applicable to DNA from Chlamydia trachomatis, and the resulting microtitration plates provide kits for the rapid clinical diagnosis of Chlamydia trachomatis infection.
Dwg.0/3

- L47 ANSWER 15 OF 19 MEDLINE
AN 93027441 MEDLINE
TI Solid-phase synthesis of oligo(2'-deoxyxylonucleotides) and PCR amplification of base-modified DNA fragments.
AU Seela F; Rosemeyer H; Krecmerova M; Roling A
CS Laboratorium fur Organische und Bioorganische Chemie, Fachbereich Biologie/Chemie, Universitat Osnabruck, FRG..
SO NUCLEIC ACIDS SYMPOSIUM SERIES, (1991) (24) 87-90.
Journal code: O8N. ISSN: 0261-3166.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9301
AB 1-(2'-Deoxy-beta-D-threo-pentofuranosyl)thymine (xTd) and -adenine (xAd) were converted into their appropriately protected 3'-phosphonates 1a, 2a as well as their 2-cyanoethyl phosphoramidites 1b, 2b. These compounds were used for solid-phase syntheses of the oligo(2'-deoxy-beta-D-xylonucleotides) 5-8. Structural properties and behavior against nucleases is described. Apart from oligo(2'-deoxyxylonucleotides) the PCR-amplification of a pUC18 DNA fragment with **Taq polymerase** was studied in the presence of the 7-deazapurine derivatives of dGTP, dATP, and dTTP. The incorporation efficiency of the modified compounds was compared with those of the parent nucleotides. 7-Deaza-2'-deoxyguanosine protected the DNA-fragment from hydrolysis by the restriction endodeoxyribonuclease Eco RI, Pst I, Bam HI, and Sma I if the nucleoside was located within the recognition site.
- L47 ANSWER 16 OF 19 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 5
AN 91:318189 BIOSIS
DN BA92:28704
TI PRODUCING SINGLE-STRANDED DNA PROBES WITH THE TAQ DNA POLYMERASE A HIGH YIELD PROTOCOL.
AU FINCKH U; LINGENFELTER P A; MYERSON D
CS FRED HUTCHINSON CANCER RES. CENT., 1124 COLUMBIA ST., SEATTLE, WASHINGTON 98104.
SO BIOTECHNIQUES 10 (1). 1991. 35-36, 38-39. CODEN: BTNQDO ISSN: 0736-6205
LA English
AB We report on efficient procedure to synthesize either single- or double-stranded probes labeled with biotin-11-dUTP, biotin-21-dUTP or digoxigenin-11-dUTP. To produce the single-stranded probe, only a single primer is utilized in a **Taq polymerase** amplification of 55 cycles. A

cytomegalovirus probe is presented. This procedure allows easy production of nonradioactively labeled pure single-stranded probes of any desired length and specificity.

L47 ANSWER 17 OF 19 SCISEARCH COPYRIGHT 1996 ISI (R)
 AN 91:52195 SCISEARCH
 GA The genuine Article (R) Number: ET526
 TI PRODUCING SINGLE-STRANDED-DNA PROBES WITH THE TAQ DNA-POLYMERASE - A HIGH-YIELD PROTOCOL

AU FINCKH U; LINGENFELTER P A; MYERSON D (Reprint)
 CS FRED HUTCHINSON CANC RES CTR, 1124 COLUMBIA ST, SEATTLE, WA, 98104; FREE UNIV BERLIN, KLINIKUM RUDOLF VIRCHOW, DEPT INNERE MED HAMATOL ONKOL, W-1000 BERLIN 33, GERMANY; UNIV WASHINGTON, DEPT PATHOL, SEATTLE, WA, 98195

CYA USA; GERMANY
 SO BIOTECHNIQUES, (1991) Vol. 10, No. 1, pp. 35.
 DT Note; Journal

FS LIFE
 LA ENGLISH
 REC Reference Count: 11

AB *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
 We report an efficient procedure to synthesize either single- or double-stranded probes labeled with biotin-11-dUTP. To

produce the single-stranded probe, only a single primer is utilized in a **Taq polymerase** amplification of 55 cycles. A cytomegalovirus probe is presented. This procedure allows easy production of nonradioactively labeled pure single-stranded probes of any desired length and specificity.

L47 ANSWER 18 OF 19 MEDLINE 90334370 MEDLINE

TI Detection of Rickettsia tsutsugamushi by gene amplification using polymerase chain reaction techniques.

AU Kelly D J; Marana D P; Stover C K; Oaks E V; Carl M
 CS Ricketsial Diseases Division, Naval Medical Research Institute, Bethesda, Maryland 20814-5055.

SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1990) 590 564-71.
 Journal code: 5NM. ISSN: 0077-8923.

CY United States
 DT Journal; Article; (JOURNAL ARTICLE)

LA English
 FS Priority Journals; Cancer Journals
 EM 9011

AB scrub typhus is commonly undiagnosed in endemic areas due, in part, to dependence on retrospective serodiagnostics. Since the etiologic agent, *R. tsutsugamushi*, will not grow in cell-free systems, a rapid direct detection system such as provided by polymerase chain reaction (PCR) methodology is needed. Genes coding for the variable 56-kDa antigen of *R. tsutsugamushi* were amplified through 35 cycles using 20-mer oligonucleotide primers and **Taq polymerase**. Amplification of 1-ng samples of DNA extracted from purified prototype *R. tsutsugamushi* Karp, Gilliam, and Kato

strains was detected by direct visual inspection of the electrophoresed, ethidium bromide-stained, specific bands. Specificity of the PCR was shown when PCR amplification of various non-scrub typhus rickettsial DNAs was unsuccessful. R. tsutsugamushi DNA extracted from the blood of infected mice could be PCR amplified and the 1477-base pair product detected by either direct visualization or by specific hybridization with amplified non-radioactive digoxigenin-11-dUTP-labeled Karp 56-kDa DNA probe.

L47 ANSWER 19 OF 19 LIFESCI COPYRIGHT 1996 CSA
AN 88:81072 LIFESCI
TI DNA sequencing using **Taq polymerase**.
AU Peterson, M.G.
CS Walter and Eliza Hall Inst. Med. Res., PO Royal Melbourne Hosp.,
Vic. 3050, Australia
SO NUCLEIC ACIDS RES., (1988) vol. 16, no. 22, p. 10915.
DT Journal
FS N; W
LA English
AB Three DNA polymerases, namely E. coli DNA polymerase 1 (Klenow), reverse transcriptase and T7 DNA polymerase (sequenase), are commonly used for DNA sequencing by the chain termination method of Sanger and colleagues. However, the secondary structure of the DNA template can impede the progress of all three polymerases. The author has developed a novel procedure in which the thermostable polymerase of Thermus aquaticus (**Taq polymerase**) is utilized in a reaction incorporating 7-deaza dGTP and performed at 70 degree C, at which the effects of secondary structure are eliminated.

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=> e brow m/au 10

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E2	2	BROW K C/AU
E3	0 -->	BROW M/AU
E4	2	BROW M A D/AU
E5	2	BROW MARK J/AU
E6	8	BROW MARY ANN/AU
E7	22	BROW MARY ANN D/AU
E8	2	BROW MARYANN D/AU
E9	2	BROW R E/AU
E10	68	BROW R K/AU

-Author (S)

=> s e4-e8; e lyamicher v/au 5

L48 18 FILE CA
L49 18 FILE CAPLUS
TOTAL FOR ALL FILES 36 ("BROW M A D"/AU OR "BROW MARK J"/AU OR "BROW MARY ANN D"/AU)
OR "BROW MARY ANN D"/AU OR "BROW MARYANN D"/AU)

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E5 10 LYAMICHEV V/AU

=> e lyamichev v/au 10

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E3 10 --> LYAMICHEV V/AU
E4 44 LYAMICHEV V I/AU
E5 2 LYAMICHEV VICTOR/AU
E6 8 LYAMICHEV VICTOR I/AU
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L52 32 FILE CAPLUS

L53 64 ("LYAMICHEV V"/AU OR "LYAMICHEV V I"/AU OR "LYAMICHEV VICTOR I"/AU)
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E7 6 OLIVE D M/AU
E8 24 OLIVE D MICHAEL/AU
E9 126 OLIVE DANIEL/AU
E10 14 OLIVE DAVID/AU

=> more

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E12 20 OLIVE DAVID L/AU
E13 2 OLIVE DEBORAH L/AU
E14 2 OLIVE DIANE/AU
E15 2 OLIVE DOMINGO JOLIS/AU
E16 2 OLIVE E/AU

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E19	2	OLIVE FERRAN/AU
E20	173	OLIVE G/AU
E21	2	OLIVE G CHAPMAN/AU
E22	2	OLIVE G HENRICI/AU

=> s e3 or e7 or e10

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L55	48	FILE CAPLUS

TOTAL FOR ALL FILES

L56	96	"OLIVE D"/AU OR "OLIVE D M"/AU OR "OLIVE DAVID"/AU
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=> s 150 and 153 and 156; s 150 and (153 or 156); s 153 and 156

L57	0	FILE CA
L58	0	FILE CAPLUS

TOTAL FOR ALL FILES

L59	0	L50 AND L53 AND L56
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L60	3	FILE CA
L61	3	FILE CAPLUS

TOTAL FOR ALL FILES

L62	6	L50 AND (L53 OR L56)
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L63	0	FILE CA
L64	0	FILE CAPLUS

TOTAL FOR ALL FILES

L65	0	L53 AND L56
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SEARCH ENDED BY USER

SEARCH ENDED BY USER

=> s 162 not (18 or 125)

L66	3	FILE CA
L67	3	FILE CAPLUS

TOTAL FOR ALL FILES

L68	6	L62 NOT (L8 OR L25)
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=> dup rem 168; d 1-3 .bevstr1; fil

biosi,medl,embas,lifesci,biotechds,wpids,confsci,dissabs,scisearch
PROCESSING COMPLETED FOR L68

L69	3	DUP REM L68 (3 DUPLICATES REMOVED)
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TI 5'-nucleases derived from thermostable DNA polymerases and their use in a nucleic acid detection method

IN Dahlberg, James E.; Lyamichev, Victor I.; Brow, Mary

Ann D.

PA Third Wave Technologies, Inc., USA

SO PCT Int. Appl., 158 pp.

CODEN: PIXXD2

PI WO 9429482 A1 941222

DS W: AU, CA, JP

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

AI WO 94-US6253 940606

PRAI US 93-73384 930604

DT Patent

LA English

AB Derivs. of thermostable DNA polymerases that retain their

5'-nuclease activity but lack polymerase are described for use in a nucleic acid detection system. The nuclease activity cleaves the single-stranded moiety of a Y-shaped structure and so is of use in selected cleavage of reporter sequences in a hybridization assay that includes two 5'-nuclease-dependent cleavage and amplification steps. The presence of the target sequence is demonstrated by the release of the reporter moiety from sequences immobilized on a carrier. The ability of the nuclease activity to cleave such structures was shown by the inability of intact Taq polymerase to amplify a hairpin sequence, although the nuclease-free Stoffel fragment could amplify the target sequence. The prepn. and characterization of a no. of polymerase mutants for use is in these assays is demonstrated.

IT Thermus aquaticus

IT Thermus flavus

IT Thermus thermophilus

(DNA polymerase of; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

IT Nucleic acid hybridization

(cleavage amplification reaction for detection of hybridization in; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

IT Deoxyribonucleic acid sequences

(for Taq polymerase lacking polymerase activity but retaining nuclease activity; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

IT Gene, microbial

RL: BSU (Biological study, unclassified); B10L (Biological study) (for Taq polymerase and polymerase-deficient analogs; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid detection method)

IT Protein sequences

(of Taq polymerase analogs lacking polymerase activity but retaining nuclease activity; 5'-nucleases derived from thermostable DNA polymerases and their use in nucleic acid

detection method)

IT 79393-91-2P, Cleavase BX
 RL: ARG (Analytical reagent use); BMF (Bioindustrial manufacture);
 ANST (Analytical study); BIOL (Biological study); PREP
 (Preparation); USES (Uses)
 (5'-nucleases derived from thermostable DNA polymerases and their
 use in nucleic acid detection method)

IT 37337-14-7P, 5'-Endonuclease
 RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation);
 ANST (Analytical study); BIOL (Biological study); PREP
 (Preparation); USES (Uses)
 (5'-nucleases derived from thermostable DNA polymerases and their
 use in nucleic acid detection method)

IT 9012-90-2D, DNA polymerase, amino acid-substituted analogs
 RL: MSC (Miscellaneous)
 (5'-nucleases derived from thermostable DNA polymerases and their
 use in nucleic acid detection method)

IT 123340-12-5 162393-95-5
 RL: ARG (Analytical reagent use); BUU (Biological use,
 unclassified); PRP (Properties); ANST (Analytical study); BIOL
 (Biological study); USES (Uses)
 (amino acid sequence; 5'-nucleases derived from thermostable DNA
 polymerases and their use in nucleic acid detection method)

IT 162393-93-3 162393-94-4 162393-96-6 162393-97-7 162393-98-8
 162393-99-9 162394-00-5 162394-01-6 162394-02-7
 RL: ARG (Analytical reagent use); BUU (Biological use,
 unclassified); PRP (Properties); ANST (Analytical study); BIOL
 (Biological study); USES (Uses)
 (nucleotide sequence; 5'-nucleases derived from thermostable DNA
 polymerases and their use in nucleic acid detection method)

L69 ANSWER 2 OF 3 CA COPYRIGHT 1996 ACS DUPLICATE 2
 AN 121:75320 CA
 TI Site-directed cleavage of nucleic acids using pilot oligonucleotides
 IN Dahlberg, James E.; Lyamichev, Victor I.; Brow, Mary
 Ann D.
 PA Wisconsin Alumni Research Foundation, USA
 SO Eur. Pat. Appl., 22 pp.
 CODEN: EPXXDW
 PI EP 601834 A1 940615
 DS R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT,
 SE
 AI EP 93-309827 931207
 PRAI US 92-986330 921207
 DT Patent
 LA English
 AB A method of cleaving a target nucleic acid mol. by use of an
 oligonucleotide with two domains is described. One of these domains
 is complementary to a sequence 5' or 3' to the cleavage site and the
 other domain is not complementary to the target DNA. Upon
 hybridization a Y-shaped complex is formed exposing the junction
 site for cleavage, e.g. with a nuclease. Suitable enzymes for
 cleaving at the junction include the thermostable nuclease

activities of DNA polymerase such as Taq, Tfl, Tth, and the gene 6 protein of bacteriophage T7. The presence of a 5'-exonuclease activity in Taq polymerase is demonstrated and the enzyme is used to cleave a PCR amplification product.

Deoxyribonucleic acids
Nucleic acids
Ribonucleic acids
RL: BIOL (Biological study)
(site-directed cleavage of, formation of single-stranded junction with pilot oligonucleotides in)
Thermus aquaticus
Thermus flavus
Thermus thermophilus

(thermostable DNA polymerase of, 5'-exonuclease activity of, for cleavage of nucleic acids at single-stranded/double-stranded junctions, formation of junctions with oligonucleotides in)
Proteins, specific or class
RL: BIOL (Biological study)
(gene 6, for cleavage of nucleic acids at single-stranded/double-stranded junctions, formation of junctions with oligonucleotides in)
Nucleotides, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC

(oligo-, forming partially single-stranded hybrids with a target nucleic acid, in site-directed cleavage)
9012-90-2, DNA polymerase
RL: USES (uses)
(thermostable, 5'-exonuclease activity of, for cleavage of nucleic acids at single-stranded/double-stranded junctions, formation of junctions with oligonucleotides in)
9025-82-5, 5'-Exonuclease
RL: USES (uses)
(thermostable, of DNA polymerases, for cleavage of nucleic acids at single-stranded/double-stranded junctions, formation of junctions with oligonucleotides in)

ANSWER 3 OF 3 CA COPYRIGHT 1996 ACS DUPLICATE 3
119:176439 CA
TI structure-specific endonucleolytic cleavage of nucleic acids by eubacterial DNA polymerases
AU Lyamichev, Victor; Brow, Mary Ann D.; Dahlberg, James E.
CS Sch. Med., Univ. Wisconsin, Madison, WI, 53706, USA
SO Science (Washington, D. C., 1883-) (1993), 260(5109), 778-83
CODEN: SCIEAS; ISSN: 0036-8075
DT Journal
LA English
AB Previously known 5' exonucleases of several eubacterial DNA polymerases have now been shown to be structure-specific endonucleases that cleave single-stranded DNA or RNA at the bifurcated end of a base-paired complex. Cleavage was not coupled

to synthesis, although primers accelerated the rate of cleavage considerably. The enzyme appeared to gain access to the cleavage site by moving from the free end of a 5' extension to the bifurcation of the duplex, where cleavage took place. Essentially any linear single-stranded nucleic acid can be targeted for specific cleavage by the 5' nuclease of DNA polymerase through hybridization with an oligonucleotide that converts the desired cleavage site into a substrate.

- IT Deoxyribonucleic acids
RL: BIOL (Biological study)
(endonucleolytic cleavage of single-stranded, by DNA polymerase-assocd. exonuclease of bacteria, 5' end structure effect on)
- IT Ribonucleic acids
RL: RCT (Reactant)
(endonucleolytic cleavage of, by DNA polymerase-assocd. exonuclease of bacteria, 5' endstructure in relation to)
- IT Bacteria
(exonuclease assocd. with DNA polymerase of, structure-specific endonucleolytic cleavage reactions of)
- IT Molecular structure-biological activity relationship
(nuclease substrate, of single-stranded RNA and DNA)
- IT 9025-82-5, 5'-Exonuclease
RL: BIOL (Biological study)
(DNA polymerase-assocd., of bacteria, structure-specific endonucleolytic cleavage reactions of)
- IT 7439-95-4, Magnesium, biological studies
RL: BIOL (Biological study)
(RNA endonucleolytic cleavage by DNA polymerase-assocd. exonuclease of bacteria dependence on)
- IT 150340-87-7
RL: RCT (Reactant)
(endonucleolytic cleavage of, by DNA polymerase-assocd. exonuclease of bacteria in primer presence and absence, 5'-end structure in relation to)
- IT 150340-89-9 150340-90-2 150340-91-3 150340-92-4
RL: RCT (Reactant)
(endonucleolytic cleavage of, by DNA polymerase-assocd. exonuclease of bacteria, 5'-end structure relation to)
- IT 9012-90-2, DNA polymerase
RL: BIOL (Biological study)
(exonuclease assocd. with, of bacteria, structure-specific endonucleolytic cleavage reactions of)
- IT 150340-88-8
RL: BIOL (Biological study)
(single-stranded DNA hairpin endonucleolytic cleavage by DNA polymerase-assocd. exonuclease of bacteria in presence of)

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=> s brow m?/au; s lyamichev v?/au; s olive d?/au

L70 16 FILE BIOSIS
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L72 10 FILE EMBASE
L73 12 FILE LIFESCI
L74 4 FILE BIOTECHDS
L75 5 FILE WPIDS
L76 0 FILE CONFSCI
L77 0 FILE DISSABS
L78 13 FILE SCISEARCH
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L80 26 FILE BIOSIS
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L84 3 FILE BIOTECHDS
L85 2 FILE WPIDS
L86 2 FILE CONFSCI
L87 0 FILE DISSABS
L88 27 FILE SCISEARCH
TOTAL FOR ALL FILES
L89 125 LYAMICHEV V?/AU

L90 325 FILE BIOSIS

L91 283 FILE MEDLINE
L92 255 FILE EMBASE
L93 81 FILE LIFESCI
L94 4 FILE BIOTECHDS
L95 8 FILE WPIDS
L96 30 FILE CONFSCI
L97 6 FILE DISSABS
L98 434 FILE SCISEARCH

TOTAL FOR ALL FILES
L99 1426 OLIVE D?/AU

=> s 179 and 189 and 199; s 179 and (189 or 199); s 189 and 199

L100 0 FILE BIOSIS
L101 0 FILE MEDLINE
L102 0 FILE EMBASE
L103 0 FILE LIFESCI
L104 0 FILE BIOTECHDS
L105 0 FILE WPIDS
L106 0 FILE CONFSCI
L107 0 FILE DISSABS
L108 0 FILE SCISEARCH

TOTAL FOR ALL FILES
L109 0 L79 AND L89 AND L99

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L111 1 FILE MEDLINE
L112 1 FILE EMBASE
L113 1 FILE LIFESCI
L114 1 FILE BIOTECHDS
L115 2 FILE WPIDS
L116 0 FILE CONFSCI
L117 0 FILE DISSABS
L118 1 FILE SCISEARCH

TOTAL FOR ALL FILES
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L121 0 FILE MEDLINE
L122 0 FILE EMBASE
L123 0 FILE LIFESCI
L124 0 FILE BIOTECHDS
L125 0 FILE WPIDS
L126 0 FILE CONFSCI
L127 0 FILE DISSABS
L128 0 FILE SCISEARCH

TOTAL FOR ALL FILES
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=> s 1119 not (119 or 146)
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 L131 1 FILE MEDLINE
 L132 1 FILE EMBASE
 L133 1 FILE LIFESCI
 L134 1 FILE BIOTCHDS
 L135 2 FILE WPIDS
 L136 0 FILE CONFSCI
 L137 0 FILE DISSABS
 L138 1 FILE SCISEARCH

TOTAL FOR ALL FILES
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=> dup rem 1139
 PROCESSING COMPLETED FOR L139
 L140 3 DUP REM L139 (5 DUPLICATES REMOVED)

=> d 1-3 bib abs; fil hom

L140 ANSWER 1 OF 3 BIOTCHDS COPYRIGHT 1996 DERWENT INFORMATION LTD

TI 5'-Nucleases derived from thermostable DNA-polymerases;
 having cleavage activity without interfering synthetic ability,
 and methods using the nucleases for detection of specific target
 sequences

AU Dahlberg J E; Lyamichev V I; Brow M A D

PA Third-Wave-Techol.
 PI WO 9429482 22 Dec 1994
 AI WO 94-US6253 6 Jun 1994
 PRAI US 93-73384 4 Jun 1993
 DT Patent
 LA English
 OS WPI: 95-036504 [05]
 AN 95-03012 BIOTCHDS
 AB A DNA sequence (I) encoding a thermostable DNA-polymerase (DP,

such that it exhibits altered DNA synthetic activity from that of the native DP is new. Also claimed are: i. a recombinant DNA vector containing (I); ii. a host cell transformed with the vector; iii. a thermostable DP altered in amino acid sequence such that it exhibits altered DNA synthetic activity from that of the native DP, but retains substantially the same 5' nuclease activity; and iv. methods for detecting the presence of a specific target DNA molecule. The new thermostable DP retains 5'-nuclease activity while the synthetic activity is reduced or absent. These new properties form the basis of a method of detecting specific nucleic acid sequences. The method relies upon the amplification of the detection molecule rather than on the amplification of the target sequence itself as do existing methods. The alteration to the native sequence is a single nucleotide deletion or insertion. Examples of DP sequences altered by deletion are specified. The

DNA is preferably derived from *Thermus aquaticus*, *Thermus flavus* or *Thermus thermophilus*. (159pp)

L140 ANSWER 2 OF 3 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN 94-185200 [23] WPIDS

DNC C94-083894

TI A method of cleaving a nucleic acid mol. at a specific target site - comprising forming a cleavage structure exposing this to a cleavage agent and incubating.

DC B04 D16

IN BROW, M A D; DAHLBERG, J E; LYAMICHEV, V I

PA (WISC) WISCONSIN ALUMNI RES FOUND

CYC 19

PI EP 601834 A1 940615 (9423)* EN 22 pp

R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

JP 06303975 A 941101 (9503) 16 pp

US 5422253 A 950606 (9528) 18 pp

ADT EP 601834 A1 EP 93-309827 931207; JP 06303975 A JP 93-339818 931207;

US 5422253 A US 92-986330 921207

PRAI US 92-986330 921207

AN 94-185200 [23] WPIDS

AB EP 601834 A UPAB: 940727

Cleaving a target nucleic acid (I) at a specific target site comprises: (a) forming a cleavage structure comprising (I) and a pilot nucleic acid (II), where a first region of (I) is annealed to (II) to form a duplex and where a second region of (I) contiguous to the duplex is not annealed to (II), forming a junction site between the duplex region and the non-annealed region; and (b) exposing the cleavage structure to a cleavage agent capable of preferentially cleaving the cleavage structure at a target site in a manner independent of the sequence of the cleavage structure; and (c) incubating the cleavage structure and cleavage agent under conditions where cleavage can occur.

USE - Using the method it is possible to manipulate nucleic acid mols. In particular a 5'-exonuclease activity of a DNA polymerase is used cleave a nucleic acid mol. The method is used to cleave a mol. in which there is some internal homology so that a part of the mol. will anneal with another part of the mol.. This is useful to detect internal sequence differences in DNA fragments without prior knowledge of the specific sequences of the variants. Dwg.0/0

ABEQ US 5422253 A UPAB: 950721

Cleavage of single-stranded nucleic acid at a specific site comprises construction of a model nucleic acid having a sequence that is complementary to that of one side of the cleavage site; prepn. of a cleavage structure of the given nucleic acid and the model, such that the model is free from any region not annealed to the given nucleic acid, but the sequence on one side of the cleavage site of the given nucleic acid is annealed to the model to form a duplex, but the sequence in the other side of the cleavage site in the given nucleic acid is not annealed to the model; and incubating the cleavage structure with either a DNA polymerase having 5'-nuclease activity, or with a gene-6-prod. from bacteriophage T-7.

USE - The process facilitates nucleic acid analysis and identification.
 ADVANTAGE - The process allows cleavage at sites containing sequences not normally cleaved by restriction endonucleases.
 Dwg. 0/10

L140 ANSWER 3 OF 3 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2

AN 93:324385 BIOSIS
 DN BA96:32735

TI STRUCTURE-SPECIFIC ENDONUCLEOLYTIC CLEAVAGE OF NUCLEIC ACIDS BY
 EUBACTERIAL DNA POLYMERASES.

AU LYAMICHEV V; BROW M A D; DAHLBERG J E

CS DEP. BIOMOLECULAR CHEM., UNIVERSITY WISCONSIN SCH. MED., 1300
 UNIVERSITY AVE., MADISON, WI 53706, USA.
 SO SCIENCE (WASHINGTON D C) 260 (5109). 1993. 778-783. CODEN: SCIEAS
 ISSN: 0036-8075

LA English
 AB Previously known 5' exonucleases of several eubacterial DNA

polymerases have now been shown to be structure-specific endonucleases that cleave single-stranded DNA or RNA at the bifurcated end of abase-paired duplex. cleavage was not coupled to synthesis, although primers accelerated the rate of cleavage considerably. The enzyme appeared to gain access to the cleavage site by moving from the free end of a 5' extension to the bifurcation of the duplex, where cleavage took place. Single-stranded 5' arms up to 200 nucleotides long were cleaved from such a duplex. Essentially any linear single-stranded nucleic acid can be targeted for specific cleavage by the 5' nuclease of DNA polymerase through hybridization with an oligonucleotide that converts the desired cleavage site into a substrate.

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L141 5 FILE CA
 L142 5 FILE CAPLUS

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L143 10 (L50 OR L53 OR L56) AND (L4 OR TAQ POLYMERASE#)
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L144 1 FILE CA
 L145 1 FILE CAPLUS

TOTAL FOR ALL FILES

L146 2 L143 NOT (L8 OR L25 OR L68)

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PROCESSING COMPLETED FOR L146

L147 1 DUP REM L146 (1 DUPLICATE REMOVED)

=> d .bevstr1; fil

biosi,medl,embas,lifesci,biotechds,wpids,confsci,dissabs,scisearch

L147 ANSWER 1 OF 1 CA COPYRIGHT 1996 ACS DUPLICATE 1

AN 113:127722 CA

TI Methods for DNA sequencing with (*Thermus aquaticus*)

DNA polymerase

IN Innis, Michael A.; Myambo, Kenneth B.; Gelfand, David H.; **Brow, Mary Ann D.**

PA Cetus Corp., USA

SO PCT Int. Appl., 29 pp.

CODEN: PIXXD2

PI WO 9003442 A1 900405

DS W: AU, JP

RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE

AI WO 89-US4093 890919

PRAI US 88-249367 880923

DT Patent

LA English

AB Methods and conditions for DNA sequencing by the dideoxy method using **Taq polymerase** are described. The high optimal temp. of this enzyme is useful for the sequencing of GC-rich DNA. The enzyme will also use secondary structure-disrupting nucleotide analogs (ITP, deazaguanine triphosphate), and the amplification products from polymerase chain reaction can be directly sequenced. Sequences >1000 nucleotides long are detd. in a single set of sequencing reactions.

IT *Thermus aquaticus*

(thermostable DNA polymerase from, dideoxy DNA sequencing using)

IT 365-08-2 1927-31-7 2056-98-6 2564-35-4 16595-02-1

101515-08-6

RL: PRP (Properties)

(as thermostable **Taq polymerase** substrate in DNA sequencing by dideoxy method)

IT 9012-90-2

RL: BIOL (Biological study)

(Taq, thermostable, DNA sequencing by dideoxy method using, elevated temp. in)

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=> s (179 or 189 or 199) and (14 or taq polymerase#)

L148 2 FILE BIOSIS
L149 3 FILE MEDLINE
L150 2 FILE EMBASE
'CN' IS NOT A VALID FIELD CODE
L151 2 FILE LIFESCI
L152 1 FILE BIOTECHDS
'CN' IS NOT A VALID FIELD CODE
L153 1 FILE WPIDS
'CN' IS NOT A VALID FIELD CODE
L154 0 FILE CONFSCI
'CN' IS NOT A VALID FIELD CODE
L155 0 FILE DISSABS
'CN' IS NOT A VALID FIELD CODE
L156 1 FILE SCISEARCH

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L157 12 (L79 OR L89 OR L99) AND (L4 OR TAQ POLYMERASE#)

=> s 1157 not (119 or 146 or 1139)

L158 1 FILE BIOSIS
L159 1 FILE MEDLINE
L160 1 FILE EMBASE
L161 1 FILE LIFESCI
L162 0 FILE BIOTECHDS
L163 0 FILE WPIDS
L164 0 FILE CONFSCI
L165 0 FILE DISSABS
L166 1 FILE SCISEARCH

TOTAL FOR ALL FILES

L167 5 L157 NOT (L19 OR L46 OR L139)

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PROCESSING COMPLETED FOR L167

L168 3 DUP REM L167 (2 DUPLICATES REMOVED)

=> d 1-3 bib abs; fil hom

L168 ANSWER 1 OF 3 LIFESCI COPYRIGHT 1996 CSA

AN 91:69900 LIFESCI

TI Methods for DNA sequencing with *Thermus aquaticus*

DNA polymerase.

AU Innis, M.A.; Myambo, K.B.; Gelfand, D.H.; Brow, M.A.D.

CS Cetus Corporation, Emeryville, CA (USA)

PI US 4075216 1991

SO (1991) . US Cl. 435/6; Int. Cl. C12P 19/34..

DT Patent

FS Q4; W; A

LA English

L168 ANSWER 2 OF 3 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 1

AN 89:161249 BIOSIS

DN BA87:83350

TI DETECTION OF ENTEROTOXIGENIC *ESCHERICHIA-COLI* AFTER POLYMERASE CHAIN REACTION AMPLIFICATION WITH A THERMOSTABLE DNA POLYMERASE.

AU **OLIVE D M**

CS DEP. MICROBIOL., FAC. MED., KUWAIT UNIV., P.O. BOX 24923, 13110 SAFAT, KUWAIT.

SO J CLIN MICROBIOL 27 (2). 1989. 261-265. CODEN: JCMIDW ISSN: 0095-1137

LA English

AB The direct identification of enterotoxigenic *Escherichia coli* from clinical specimens was examined by using the polymerase chain reaction (PCR) for amplifying the heat-labile toxin (LT) gene. Two synthetic primers, each of which was 20 bases in length, were used with the thermostable DNA polymerase from *Thermus aquaticus* to amplify the LT gene. The amplified PCR products were detected by either gel electrophoresis or hybridization to a 24-base synthetic oligonucleotide probe conjugated to alkaline phosphatase. The PCR method detected LT-positive bacteria but did not react with *E. coli* producing the heat-stable toxin, enteroinvasive *E. coli*, *Salmonella typhi*, *Salmonella typhimurium*, or *Shigella dysenteriae*. By the PCR method, a single bacterium could be detected following 30 cycles of amplification. The *T. aquaticus* DNA polymerase was inhibited by more than 103 organisms in the amplification reaction mixture. A group of 40 clinical specimens consisting of 16 LT bioassay-positive and 24 LT bioassay-negative stool specimens were tested by PCR for the presence of toxigenic *E. coli*. The total DNA from 100 .mu.l of stool specimen was extracted and partially purified with a commercially available ion-exchange column. All 16 of the bioassay-positive stool specimens were positive by PCR. In addition, one stool specimen which was bioassay negative for LT but positive for LT in a previous hybridization assay with a

different LT probe was also positive by PCR. This may indicate that the LT gene is present but either is not expressed or is expressed below detectable levels. Amplification of specific DNA sequences by PCR provides a highly sensitive and specific tool for the detection of pathogenic microorganisms directly from clinical specimens without the need for prior isolation. This technique may find wide application in the detection of other organisms in addition to enterotoxigenic E. coli.

L168 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 1996 ISI (R)

AN 89:4354 SCISEARCH
GA The genuine Article (R) Number: R5187
TI DNA SEQUENCING WITH THERMUS-AQUATICUS DNA-
POLYMERASE AND DIRECT SEQUENCING OF POLYMERASE CHAIN
REACTION-AMPLIFIED DNA

AU INNIS M A (Reprint); MYAMBO K B; GELFAND D H; BROW M A D
CS CF MENNINGER MEM HOSP, DEPT MICROBIAL GENET, 1400 53RD ST, TOPEKA,
CVA USA KS, 66601 (Reprint)

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES
OF AMERICA, (1988) Vol. 85, No. 24, pp. 9436-9440.

DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 19

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E3	0 -->	7-DEAZA-DATP/CN
E4	1	7-DEAZAADENINE/CN
E5	1	7-DEAZAADENOSINE/CN

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